The Journal of Allergy

VOL. 5 JULY, 1934 NO. 5

Original Articles

THE DEVELOPMENT OF HYPERSENSITIVENESS IN MAN

I. FOLLOWING INTRADERMAL INJECTION OF THE ANTIGEN*

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IT IS generally recognized that the parenteral introduction of a foreign serum into the human being is followed by the development of hypersensitiveness. When the quantities of serum are large, serum disease often occurs, which in turn is followed by the development of positive skin tests, and often by the appearance of specific antibodies, precipitins and anaphylactins in the blood.[1] But even when the quantities are very small, certain evidences of hypersensitiveness may appear. Hooker[2] and also Park[3] have shown that such small quantities of horse serum as are present in the Schick diphtheria toxin-antitoxin treatment mixture are sufficient to change the organism so that later on a skin test with horse serum will give a positive result. Tuft[4] also finds that 27.9 per cent of children develop a skin test to horse serum after the Schick treatment.

The development and the symptoms of hypersensitiveness vary considerably among the various animal species. While the differences may be largely quantitative, it is evident that experiments on laboratory animals can provide only general suggestions concerning the development of clinical allergy in man. It seemed desirable, therefore, to study the development of sensitiveness as determined by the results of skin tests and other phenomena in man himself. It was important to see whether this development would be different in those individuals called "atopic" because they suffered from asthma or hay fever from

*From the Anaphylaxis Clinic of the Massachusetts General Hospital.
Aided by the M. G. H. Asthma Fund.
Read before the Tenth Annual Meeting of the Society for the Study of Asthma and Allied Conditions, Atlantic City, N. J., April 28, 1934

those others called "normal" or "nonatopic." A third object was to find out whether the hypersensitiveness induced artificially by repeated injections of small amounts of foreign serum was the same as or different from the other hypersensitiveness which occurs "naturally" in patients with hay fever, asthma, or eczema.

While this work was in progress, the very important paper by Jones and Mote[5] has appeared. In attempting to obtain physiologic breakdown of streptococci, Jones and Mote injected large amounts of living organisms into the peritoneal cavity of immune rabbits. Three hours later they removed and filtered the peritoneal fluid which they hoped would contain specific bacterial substances in soluble form. This fluid was tested intradermally on patients with arthritis, and a high percentage of positive results was obtained. Later, Jones and Mote found that the fluid of normal rabbits would give almost as many reactions, and they concluded that the reactions were due to the rabbit rather than to the bacteria. They observed that when first tested, few patients reacted, but after several doses, many patients showed a twenty-four-hour inflammatory reaction of the tuberculin type. Still later, when the series of intradermal injections was continued, reactions of the immediate urticarial type began to appear. The two types of local reaction represented different phases of the immune process. This work, like our own, is quite in line with the observations of Dienes[6] on the skin reactions which develop in guinea pigs following repeated intracutaneous injections of foreign serum and of egg white.

Our plan was to study the effect of repeated doses of foreign serum injected intracutaneously in small quantities at weekly intervals. Guinea pig serum was selected because it is a protein antigen rarely concerned with ordinary life. Dilutions were made with buffered saline solution, and the volume injected intracutaneously was always 0.1 c.c. of the diluted serum. The subjects to whom the injections were given, comprised 46 "atopic" patients from the Anaphylaxis Clinic who were under treatment for hay fever, asthma, vasomotor rhinitis, or atopic eczema, and a group of 25 "nonatopic" or "normal" individuals, comprising 22 patients with arthritis or with chronic diseases in the hospital wards, and three doctors. Following each dose, every patient and every control individual was observed in the clinic for at least twenty minutes and usually for half an hour in order to see whether an immediate urticarial reaction would develop. The study of delayed reactions occurring in twenty-four hours was less satisfactory. Patients on the wards, the doctors, and a few outpatients could be observed for several days in succession so that their reactions could be observed directly, but in the case of other outpatients who could not return to the clinic, reliance was placed on the

individual's statements of the presence or absence of any redness or swelling at the site of inoculation.

Early in the study it became evident that both atopic individuals and normal persons varied somewhat in their responses to the first one or two doses. Interpretation was often difficult. In each case, the intracutaneous injection itself produced at once a mechanical wheal from 8 mm. to 10 mm. in diameter. In most of the cases, this subsided rapidly. In some, however, the mechanical wheal increased in size, and five or ten minutes later became surrounded by a red zone of varying width. In a few cases, this increase continued until at the end of fifteen minutes, the wheal and flare became "typical" and was quite comparable to the response which the hay fever patient gives to specific pollen extract. In this study, we have restricted the term "immediate reaction" to this "typical" wheal and erythema response.

We were rather surprised at the outset to find that many of the hay fever patients gave positive immediate skin tests to guinea pig serum on the first intracutaneous dose whether of the 1:10 or of the 1:100 dilution, and this occurred even when the volume injected was reduced to 0.02 c.c. or even to 0.01 c.c. In asthmatic individuals and in normal persons, however, this tendency to give immediate skin reactions to the first intradermal injection of guinea pig serum was less pronounced. Many of these could tolerate the 1:10 dilution without showing any increase of the usual mechanical wheal. In contrast to the immediate reactions just described, delayed reactions appearing in twenty-four hours after the test dose and comparable in appearance to the tuberculin reaction, were rarely observed after the first injection. Occasionally the first dose did result in a small red inflammatory area. Following the later doses, however, delayed reactions were commonly observed as red, slightly swollen areas, rarely with pale centers, which varied in size from 10 mm. to 40 mm. in diameter. These appeared in eighteen or twenty-four hours and lasted from one to four days, usually fading to a discoloration which after strong reactions might persist for a week.

In the study of results, the patients may be divided into groups according to the dilution of guinea pig serum used in their treatment.

Sixteen atopic patients received repeated intracutaneous doses (0.10 c.c. each time) of 1:10 guinea pig serum (Table I). Normal, nonatopic individuals were not studied with this strong material. Sensitiveness was produced almost at once and with regularity. One dose was usually sufficient to sensitize so that the second dose injected a week later resulted in a delayed response, and in five of the cases this response was well marked (XX). Immediate reactions were observed in only three patients after the first dose, but later in the course

of study, immediate reactions were observed in each and every case, frequently following the third dose, more often after the fourth and fifth doses. In several instances, the immediate wheals were of considerable size—even up to 25 mm. in average diameter. Table I shows the details and time relations, but it does not show an additional fact, that in most of the cases delayed reactions were observed on the next day when the immediate response had subsided. The table indicates clearly enough that repeated intracutaneous injection of a 1:10 dilution of guinea pig serum results in the prompt and regular development of first delayed and then immediate skin reactions.

TABLE I

REACTIONS TO REPEATED INTRACUTANEOUS INJECTIONS, 0.10 C.C. EACH, OF GUINEA PIG SERUM **Diluted 1:10** IN ATOPIC PATIENTS

	PATIENT	AGE	DIAGNOSIS	WEEKS															
				0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1.	T. W.	26	Asthma V. M. R.	O	X	X	X	X	(13)	(16)	(19)									
2.	J. S.	25	Asthma	O	X	XX	(18)	(22)												
3.	S. D.	47	Asthma	O	X	XX		XX	(11)		(15)		(18)							
4.	M. B.	52	Asthma	O	X	XX		XX	(12)	(9)	(18)	(13)	(16)	(13)		(17)				(16)
5.	L. W.	46	Asthma	(11)	(9)	(8)	(9)	(7)		(11)				(13)				(15)		
6.	H. B.	12	V. M. R.	O	O	O	O	O	O	O	(13)									
7.	L. N.	43	Asthma	O	XX		(16)	(15)	(15)				(25)							(15)
8.	J. L.	35	Asthma	O	XX		(12)	(11)	X	XX	(12)		(11)					(13)		
9.	E. L.	23	Atopic eczema	O		O		O	(10)	(9)		(13)		(15)		(16)				
10.	G. F.	50	Asthma	O	O	X	X	X	(12)	(10)	(14)	(17)	(16)							
11.	F. B.	48	Asthma	O	X	XX		XX	(12)				(10)	(10)	(11)	(12)		(16)		
12.	J. P.	20	V. M. R.	(10)	(11)	(13)		(12)	(13)				(15)					(17)		
13.	S. D.	21	Asthma	X	XX	XX	(20)	(25)	(20)		(17)									
14.	S. E.	54	Asthma	O	XX	XX				O				XX			(9)	(11)	(13)	
15.	M. O.	48	Asthma	O	XX	(10)	XX	(10)												
16.	H. S.	22	H. F.	(10)				(11)			(20)									

In this and the following tables, delayed reactions are expressed as O, X or XX according to the patient's story or as actual measurements in millimeters if observed by us. Immediate reactions are expressed by figures inside of circles, the figures indicating the average diameter of the wheal in millimeters.

The degree of sensitiveness in these patients was studied. The total number of injections received by each patient varied from three to twelve. At the end of the treatment, intracutaneous tests with serial dilutions of guinea pig serum were made and showed considerable variation among the members of the group. The least sensitive patients reacted to no higher dilution than 1:1,000, while the most sensitive patient gave a definite wheal and erythema with a 1:100,000 dilution intracutaneously (0.02 c.c.), and to a 1:100 dilution by the scratch method. Control tests with diluting fluid were, of course, made in every case. The most sensitive patient was, therefore, one hundred times as sensitive as the least sensitive, as determined by the immediate skin test.

The duration of sensitiveness was studied in five patients who were followed for from five to twelve months. In one case, a well-defined wheal to the 1:1,000 dilution disappeared so that even the 1:10 dilution no longer gave any response; but in the other four cases, tests with the 1:1,000 dilution, even after an interval of several months, still showed a positive reaction.

TABLE II

REACTIONS TO REPEATED INTRACUTANEOUS INJECTIONS, 0.10 C.C. EACH, OF GUINEA PIG SERUM **Diluted 1:100** IN NONATOPIC AND ATOPIC PERSONS

	PATIENT	AGE	DIAGNOSIS	WEEKS											
				0	1	2	3	4	5	6	7	8	9	10	
				Nonatopic Individuals (Controls)											
1.	E. S.	54	Arthritis	1	3	7									
2.	P. P.	22	Old Polio*	7	14	18	22	35	12						
3.	J. H.	31	Arthritis	10	15	22									
4.	E. C.	24	Arthritis	12	14	20	22								
5.	L. P.	19	Old Polio	10	18	14	17								
6.	L. L.	20	Old Polio	11	15	22									
				Atopic Patients											
7.	M. C.	40	Asthma	O	X	XX	XX	XX	(12)	(14)					
8.	A. C.	31	H. F.	(12)	(12)	(14)	(14)			(13)					
9.	R. B.	32	V. M. R.	O	O		O	X			X	X			
10.	G. G.	35	Asthma	O		O	X?	X?	O	O			O		1–10 X
11.	H. S.	37	H. F.	O	O	O	X	X	X	X		(13)			
12.	N. O.	47	Asthma	O	O	XX	XX		XX			(9)			
13.	J. O.	17	Asthma	O		O		O	O	XX	(12)		(14)		
14.	R. L.	29	Asthma	O	O	X	X	(12)	(13)	(12)					
15.	F. R.	34	H. F.	O	X	XX									
16.	T. C.	36	V. M. R.	O	O	XX	XX	XX	XX	X	(11)				
17.	J. S.	36	Asthma	O	O	X?	O		11			O			
18.	M. D.	42	H. F.	(15)	(12)	(12)	(12)	(14)	(15)	(12)					
19.	M. M.	29	H. F.	(10)	(9)	(10)	(12)	(10)	(11)						

*Old poliomyelitis observed in orthopedic ward.

The 1:100 dilution was used in 6 nonatopic persons and 13 atopic patients (Table II). Delayed reactions occurred in all except one (Case 10) and after a very few doses, and even this case reacted with a delayed reaction some weeks later when tested with the 1:10 dilution. Immediate reactions developed in six of the cases, but they developed much later in this experiment than when the 1:10 dilution was used. Three patients showed immediate reactions after the first dose, and all of them had symptoms of hay fever. Whether the development of delayed or of immediate reactions was more marked in the atopic than in the normal or nonatopic individuals is doubtful. The number of cases is too small for any close comparison. Table II indicates, however, that the time intervals and reaction sizes were about the same in the two groups. Incidentally, it should be noted that patients did not always return to the clinic each week. The table shows that even with the 1:100 dilution, the individual is easily

changed so that he reacts in a new and characteristic fashion after a comparatively few doses.

Twenty-seven persons were injected intracutaneously repeatedly with the 1:1,000 dilution. Table III shows that delayed reactions occurred with about equal frequency among the normal and the atopic individuals and after about the same number of doses. It is interest-

TABLE III

REACTIONS TO REPEATED INTRACUTANEOUS INJECTIONS, 0.10 C.C. EACH, OF GUINEA PIG SERUM **Diluted 1:1000** IN NONATOPIC AND IN ATOPIC PERSONS

	PATIENT	AGE	DIAGNOSIS	WEEKS												
				0	1	2	3	4	5	6	7	8	9	10	11	12
			Nonatopic Persons (Controls)													
1.	Dr. J. R.	30	Not sick	7	5	10	10	20								
2.	Dr. F. R.	45	Not sick	5	5	4	6	6	9	(0)	(0)	(0)	(0)			
3.	Dr. J. D.	28	Not sick	5	9	9	10	20	15							
4.	A. S.	49	Arthritis	2	10	15										
5.	C. P.	46	Arthritis	2	3	4	2	3	4	5	7	4				
6.	M. D.	28	Arthritis Old Polio	8	3	3	2	3							(0)	
7.	W. D.	14	Cong. Dislocation of Hip	10	10	16	10	14								
8.	C. C.	42	Sacroiliac strain	3	3	3	3								(0)	
9.	W. H.	16	Osteomyelitis	3	3	3	5	20	4*	20						(0)
10.	A. F.	27	Tb. of Hip	3	2	2	5	6	12				(0)			
11.	R. H.	38	Tb. of Spine	4	6	10	13	15	10	9						
12.	D. M.	34	Osteomyelitis	4	3	5										
13.	O. F.	32	Tb. of Spine	13	12	18	20									
			Atopic Patients													
14.	F. S.	25	Asthma	3	2	4	4	5	22	23						
15.	C. S.	24	V. M. R.	3	2	3	20									
16.	C. H.	38	Atopic eczema	4	3	11	11	12		(0)						
17.	P. B.	48	Asthma	O	O	O	O	X								
18.	G. B.	37	Asthma	O	O	O	X	XX	X							
19.	I. E.	21	Asthma	O	O	O	X	X								
20.	H. M.	22	Atopic eczema	O		O		O		X	X					
21.	R. D.	41	H. F. and asthma	(17)	(15)	(14)	(16)	(14)	(12)							
22.	P. H.	12	Asthma	O	O	X	X	(14)	(10)	(12)	(11)					
23.	R. K.	43	Asthma	O	O	O	O	O								
24.	G. K.	26	V. M. R.	O	O	O	O									
25.	M. W.	42	H. F.	O			O	O	O	O	X					
26.	J. O.	37	Asthma	O	O	O	O									
27.	M. B.	45	H. F.	(17)	(14)	(12)	(11)		(12)	(13)						

*Surgical operation that day

ing that even after this very mild treatment, delayed reactions should reach a size of 20 mm. after the third or fourth dose. Immediate reactions after the first dose were observed in two patients who had hay fever, and thereafter in these individuals immediate reactions occurred after each and every dose. The treatment was continued simply to see whether any change would develop. Table III shows no change in the immediate reaction, but it does not show an interesting observation, that in Cases 21 and 27 delayed reactions also occurred after the third and all subsequent doses. Immediate reactions which de-

veloped under observation and as a direct result of the treatment were observed in only two cases; one after the fourth, the other after the sixth treatment. Once developed, these reactions persisted for several weeks, but in one patient tested after twenty weeks, the reaction was distinctly smaller. In five instances, tests for immediate reactions were made many weeks after the original treatment in order to see whether time alone would not lead to a positive test. The results, however, were all negative.

In this study, the comparison of the delayed or tuberculin type of skin reaction with the immediate or urticarial type reaction is fundamental. Our results confirm the findings of Dienes and of Jones and Mote in every particular.

The tuberculin type reaction occurs in the early stages of the development of hypersensitiveness. How early? In a special extra series of nine cases treated with the 1:10 dilution, a delayed reaction was elicited by a second intradermal injection given as soon as three days after the primary or sensitizing injection which itself had produced no reaction. In one patient to whom the second injection was given eight days after the first, the delayed reaction did not occur within twenty-four hours as usual but appeared four days later as a "flare" reaction; thus indicating that in this case, an incubation period of twelve days was required for the development of hypersensitiveness. Evidently, a single first dose is quite sufficient to sensitize, and the development of sensitiveness may be rapid. The largest amount of treatment needed to produce a delayed reaction was two doses, each of 0.10 c.c. of the 1:10 dilution representing in all 0.02 c.c. of serum. (See Table I, Case 10.) The smallest amount was a single dose containing 0.10 c.c. of the 1:1,000 dilution representing a 0.0001 c.c. of serum. (See Table III, Case 4.)

The immediate urticarial reaction appearing in a few minutes after the test dose is a late phase of hypersensitiveness. All the patients treated with the 1:10 dilution developed it but usually not until from three to five weeks after the first injection. It occurred as early as the third week in some cases and as late as the thirteenth week in one case.

In those patients treated with the 1:100 dilution, the hypersensitiveness developed more slowly so that both the delayed and the immediate reactions appeared later. Many of the patients injected with this dilution were not under treatment and observation over a period of time long enough for the hypersensitiveness to develop to such a point that the immediate reaction could be elicited with serum of 1:100 dilution. It did develop, however, in several cases. Even when a dilution as high as 1:1,000 was used in treatment, definite sensitiveness was produced and immediate reactions were observed at least twice.

A summary of all the findings appears in Table IV.

TABLE IV

DILUTION OF SERUM	NORMAL OR NONATOPIC PERSONS			ATOPIC PATIENTS		
	TOTAL	SENSITIVE	NOT SENSITIVE	TOTAL	SENSITIVE	NOT SENSITIVE
1:10	—	—	—	16	16	0
1:100	6	6	0	13	12	1
1:1000	13	8	5	14	11	3

The question of local skin-area hypersensitiveness has been investigated. A group of nine patients was given two intracutaneous injections at each visit. One of these was always in the same spot. The other was in a previously untreated area. Delayed reactions occurred in all nine patients and were of approximately the same size and character in the reinjected area as in the area tested for the first time. They appeared no sooner in the reinjected area than elsewhere. In the case of immediate urticarial reactions observed some time later, one of the subjects showed a very definite difference between the responses of the particular spot and the skin generally. On several occasions, the test doses given to this individual caused a wheal in the particular spot and no reaction elsewhere. In most cases, however, it appeared true that the entire skin surface of the body becomes sensitized simultaneously. This result is at some variance with the finding of Redfern who reinjected 0.03 c.c. of undiluted rabbit serum intracutaneously into the same area in ten patients and obtained evidence of much greater sensitization in the reinjected area than in another area tested at the same time.

Passive transfer experiments were made in twelve patients all in the 1:10 group at times varying from two weeks to six months after the start of treatment. In two instances, there was a suggestion that transfer had taken place but the reaction was doubtful at best. In a third case, however, a definite reaction was obtained in two separate recipients when the sites prepared with the patient's serum were reinjected on the third day with the 1:10 and the 1:100 dilutions of guinea pig serum. Control areas prepared with normal human serum were negative. Specificity was shown by negative reactions to egg white and diluting fluid. It is important to note, however, that this patient was the most sensitive of the entire group. She gave positive scratch tests to the 1:100 dilution of guinea pig serum and positive intradermal tests to the 1:100,000 dilution. One cannot escape the suggestion that passive transfer with the serum of other patients might have been successful if hypersensitiveness in these cases had developed to a sufficiently high degree. A corollary to this thought is that the difference between our subjects artificially sensitized by intracutaneous doses and our patients naturally sensitive to foreign substances

of various kinds may be merely a difference in degree. All efforts to demonstrate precipitins and complement fixing antibodies gave negative results.

The flare reaction or "local serum sickness" was observed in three of the 71 cases. In the first case, the second injection was followed by a delayed reaction lasting six days. On the day of the third injection, the site of the second, which had almost disappeared, became red and slightly swollen and remained so for two days. The third injection gave an ordinary delayed reaction beginning the following day and lasting one day, so that both the second and the third faded at the same time.

In the second case, the second injection was followed by a delayed reaction lasting five days. Two days following the third injection, given seven days after the second, and fourteen days after the first, all three injected areas became red and swollen, and remained so for five to six days, gradually fading out.

In the third case, the second injection given eight days after the first, remained entirely negative for four days, then became red and swollen and remained so for two days.

One theoretical explanation for these flare reactions is that a small quantity of antigen remains fixed in the tissues of the injected area. Sensitization develops uniformly and simultaneously throughout the entire skin area of the body. When it reaches a certain level, there still remains in the injected area a concentration of antigen sufficient for the reaction between it and the tissue antibodies to result in a visible inflammatory response. Absence of a flare would be explained by a more rapid absorption of antigen or by a more slowly developing sensitization (local tissue antibodies) or both.

DISCUSSION

That a hypersensitiveness to foreign serum demonstrable by skin test can be induced in man easily and regularly by one or more doses injected intracutaneously has been well established. Hooker and also Park induced it with horse serum, Jones and Mote have induced it with rabbit serum, and the present experiments show successful results with guinea pig serum.

The data show that the degree of this induced sensitiveness may be high or low in spite of treatment which is always the same. Individual differences among the normal persons and among the atopic patients tested occur, but as for any striking difference between the group called "nonatopic" or "normal" and the other group called "atopic," the data indicate that it does not exist. The reactions are closely similar in the two groups.

The amount of treatment is important. Sensitiveness develops more promptly and in more subjects when the stronger 1:10 dilution of serum is used. It is interesting, however, that even with the 1:1,000 dilution, repeated treatment is quite capable of inducing a characteristic response. It is unfortunate that the experiment could not have been continued indefinitely or at least long enough to induce an immediate wheal and erythema reaction in each case. We believe, however, that the data now at hand justify the thought that this ideal will ultimately be attained.

Is the hypersensitiveness produced artificially in these experiments the same as or different from the hypersensitiveness of patients with hay fever and asthma? The question is hard to answer. It can be said: First, that all the skin is sensitive, at least in most cases. Whether tissues other than the skin are sensitive remains to be seen. Second, antibodies are present in the blood so that the serum of artificially sensitized persons is able to transfer the sensitiveness to a normal site in the skin of a normal recipient. It should be observed, however, that passive transfer is not possible unless the degree of sensitiveness is such that a skin test with dilute serum results in a well-marked wheal and erythema reaction. We asume that the presence or absence of antibody demonstration (reagins) rests on a quantitative rather than a qualitative basis.

The degree of sensitiveness is less in our subjects than in others naturally sensitive. All attempts to increase the degree of artificial sensitiveness by repeated injections carried out for several months have failed. The high degree of sensitiveness characteristic of patients with hay fever and asthma has not been produced. So far, we have not seen any excessive local reactions, nor have any general symptoms followed any of the intracutaneous doses. The difference is real. Whether the difference depends upon some unknown factor or whether it rests upon a quantitative rather than a qualitative basis is unknown.

The persistence of the skin reaction may be important. In hay fever and asthma it is characteristic for the skin test to persist for a long time after all clinical sensitiveness has subsided. Whether it persists in artificial sensitiveness is doubtful. It did so in four out of five cases, but the number is too small to be conclusive.

SUMMARY

1. Repeated intracutaneous injections of guinea pig serum into man will sensitize him so that a subsequent injection will produce at first a delayed reaction of the tuberculin type and later an immediate reaction of the wheal and erythema type.

2. Artificial sensitiveness results more rapidly and more regularly from doses of the 1:10 dilution than from doses of the 1:100 or 1:1,000 dilutions.

3. Artificial sensitiveness is produced in nonatopic, normal persons to about the same extent and with about the same ease as in atopic patients.

4. Antibodies capable of transferring the sensitiveness locally to the skin of a normal recipient may be demonstrated when the degree of sensitiveness, as measured by skin test, is large but not otherwise.

5. "Flare" reactions are sometimes observed.

6. The relation between this artificial sensitiveness and natural sensitiveness is discussed.

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DISCUSSION

DR. F. M. RACKEMANN.—I should like to emphasize one point and that is that success in sensitizing these normal and atopic individuals appears to be proportional to the strength of the materials used. My personal opinion is that sensitization is simply a reaction of the normal person to this treatment; that is to say, that sensitiveness can be produced in perfectly normal people, without other factors, and that the difference between normal and atopic persons is, after all, simply a difference in degree.

DR. MARION B. SULZBERGER, New York City.—I think that one should make a further remark on this interesting contribution. Dr. Simon has shown that it is possible to achieve experimentally a high percentage of human sensitization with the serum of an animal with which there has been no possibility of prior contact. This sensitization is apparently equally successful in atopic and in nonatopic individuals. However, the sensitization differs in many respects from an atopic hypersensitivity; for instance, there are generally no reagins. According to the records of those who have used foreign serums extensively in therapy, there are no shock reactions in this type of nonatopic sensitization. This seemed astonishing to me.

I should expect that an atopic person would be much easier to sensitize than a nonatopic one, even with a foreign serum. It is particularly interesting when an experiment turns out to be the opposite of our expectations, as Dr. Simon's has done. It seems to me that the

result means that even in an atopic individual there must be another factor at play which makes him or her become atopically hypersensitive to a given substance at a given time. It is not necessarily just contact with that substance that does it. That is what I think Dr. Simon and Dr. Rackemann wanted to bring out. We may on this path find the explanation as to why one atopic individual becomes sensitive to ragweed, and another to horse dander and another to something else. Perhaps the factors underlying the creation of an atopic hypersensitivity are not constantly but sporadically operative. For instance, and hypothetically, an atopic child weaned and, therefore, upset, during the ragweed pollenization season, may become sensitized to ragweed, while another child in the same family, weaned in the spring may become sensitized to timothy or spring grasses.

It is conceivable that only those contacts sensitize atopically which are established during the period of operation of the underlying factors.

THE DEVELOPMENT OF HYPERSENSITIVENESS IN MAN

II. Absorption of the Antigen Through the Nasal Mucous Membrane*

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IN A PREVIOUS publication[1] we described experiments by which it was shown that hypersensitiveness in man may easily and regularly be produced by the intracutaneous injection of small amounts of guinea pig serum. Since this method of introduction of antigen into the body is such an unnatural portal of entry for those substances responsible for hay fever and asthma, the nasal mucous membrane as a portal of entry for antigen in man appeared to be worthy of further study.

There is abundant evidence that sensitization through the nose can take place. The development of sensitiveness to a great variety of substances met with in industry must inevitably be by this route. Castor bean dust, orris powder, insect powder, grain dust, and other substances have produced hypersensitiveness in persons exposed to them. In the case of castor bean, the report of Figley and Elrod[2] that so many factory workers developed asthma as to constitute almost an epidemic is interesting from many points of view. More experimental is the demonstration by M. B. Cohen et al.[3] that the reactive substance in ragweed pollen could pass through the intact nasal mucosa.

In laboratory animals, hypersensitiveness may be readily produced by dropping or spraying the antigen into the nose, and interesting experiments are reported by Sewell and Powell,[4] Ratner, Jackson, and Gruehl,[5] Arloing and his associates,[6] and by Alexander, Becke, and Holmes.[7]

Our success with the intradermal route gave us confidence that it would be easy to sensitize human subjects by applying guinea pig serum directly to the nose. Nine patients who suffered from hay fever, chronic vasomotor rhinitis or asthma were selected at a time when they were essentially free of nasal symptoms. Guinea pig serum in 1:10 dilution with carbolized (0.50 per cent) buffered saline was applied to the nasal mucous membrane by means of a flat piece of absorbent cotton two or three inches long, saturated with the diluted serum and inserted into the nasal cavity. This nasal pack was allowed to remain in place for from fifteen to thirty minutes. A series of applications varying from three to six was made in this manner. Control applications of

*From the Anaphylaxis Clinic of the Massachusetts General Hospital.
Aided by the M. G. H. Asthma Fund.

diluting fluid were frequently made on the opposite side at the same time or at another time. The time interval between applications varied from three days to two weeks or longer. In Case 4, five and one-half months elapsed between the third and fourth applications.

The first application produced no nasal symptoms in any of the patients, but following the second, third, or subsequent applications there

TABLE

RESULTS OF APPLICATIONS OF

CASE NO.	CLINICAL EXPRESSION OF ALLERGY	FIRST NASAL PACK			SECOND NASAL PACK			THIRD NASAL PACK		
		DATE	TYPE	NASAL SYMP.	DATE	TYPE	NASAL SYMP.	DATE	TYPE	NASAL SYMP.
1	Ragweed hay fever	6/12/33	R:S	0	6/26/33	R:S	+	7/ 3/33	R:S L:D	+
2	Ragweed hay fever	6/12/33	R:S	0	6/26/33	R:S	+	7/ 3/33	R:S L:D	+
3	Ragweed hay fever	6/12/33	L:S	0	6/19/33	L:S	+	7/ 3/33	R:D	±
4	V. M. R.	3/31/33	R:S	0	4/14/33	R:S	±	5/22/33	R:S L:D	+
5	Ragweed hay fever	7/20/33	R:S L:D	0	7/24/33	R:S L:D	0	7/31/33	R:S L:D	0
6	Ragweed hay fever	7/24/33	R:S L:D	0	7/31/33	R:S L:D	0	8/ 7/33	R:S L:D	0
7	Asthma	5/26/33	L:S	0	6/ 2/33	L:S	0	6/ 9/33	L:S	0
8	V. M. R.	5/22/33	R:S	0	6/16/33	R:S	0	7/21/33	R:S L:D	+
9	V. M. R.	3/24/33	R:S	0	3/31/33	R:S	0	4/14/33	R:S	0

R = right side.
L = left side.
S = guinea pig serum 1:10.

were nasal symptoms in four cases which could be definitely attributed to the serum. The symptoms usually began within from six to twenty-four hours, occasionally in from ten to fifteen minutes. They lasted from two to six days and consisted of nasal obstruction, thin serous discharge, and often sneezing. In most instances the patient could detect a definite difference between the two sides when serum was put into one side and diluting fluid into the other, the symptoms being worse on the side having the serum or limited entirely to this side. In three of these patients, the fourth nasal pack consisted of diluting fluid only. There were no symptoms following this treatment. The fifth pack in the same three patients consisted of serum on one side and diluting fluid on the other. There were definite nasal symptoms following this treatment. Three patients had no nasal symptoms whatever following applications of serum. These patients were clinically indistinguishable from those just described who had very definite symptoms.

The cause of this difference is not apparent. In two cases, nasal symptoms following applications of serum were very indefinite or irregular in their occurrence so that they could not definitely be ascribed to the serum. Table I summarizes the results.

A single intracutaneous test with guinea pig serum in varying dilutions of 1:10 to 1:1,000,000 was made at some time during the course

I

SERUM TO NASAL MUCOUS MEMBRANE

FOURTH NASAL PACK			FIFTH NASAL PACK			SIXTH NASAL PACK			FIRST SKIN TEST		SECOND SKIN TEST	
DATE	TYPE	NASAL SYMP.	DATE	TYPE	NASAL SYMP.	DATE	TYPE	NASAL SYMP.	DATE	RESULT	DATE	RESULT
7/17/33	R:D	0	7/31/33	R:S L:D	+				8/ 7/33	Imm.		
7/17/33	R:D	0	7/24/33	R:S L:D	+				7/31/33	Imm.		
7/17/33	L:D	0	7/24/33	R:S L:D	+				8/10/33	Imm.	8/14/33	Imm.
11/ 6/33	R:S L:D	+							4/21/33	Del.	5/15/33	Imm.
8/ 3/33	R:S L:D	0	8/ 7/33	R:S L:D	0	8/14/33	R:S L:D	0	8/21/33	Imm.	8/28/33	Imm.
7/17/33	L:S R:D	0	7/31/33	L:S R:D	0	8/ 4/33	L:S R:D	+	6/16/33	Del.	6/23/33	Imm.
8/ 3/33	R:D L:S	±							6/23/33	Del.	6/30/33	Imm.
									4/21/33	Del.	4/28/33	Imm.

D = dilating fluid.
Del = delayed reaction.
Imm = immediate reaction.

of the observation period, early in some cases and later in others. Hypersensitiveness of the skin developed in eight of the nine cases, apparently as a result of the intranasal treatment. The ninth patient did not return for skin tests. The reactions were of the delayed type in those tested from three to four weeks after the first nasal application. Immediate reactions occurred in those tested from five to eight weeks following the first application of serum. These results show that sensitiveness manifested by positive skin reactions may be produced by absorption through the nose as well as by injection into the skin.

It is of interest to compare skin reactions with nasal symptoms. In the first few weeks of a developing hypersensitiveness, delayed skin reactions are obtained. These last often for several days. At this period nasal symptoms parallel very closely these delayed skin reactions. They begin the day following the application of serum and last for several days. After five or six weeks have elapsed since the first

application of serum, the skin reactions become "immediate." Nasal symptoms following the intranasal application of serum at this time may have an early onset. In several instances, symptoms began in from ten to fifteen minutes, the nasal pack being expelled by violent sneezing and copious discharge.

The experiment indicates that hypersensitiveness of the skin and of the nasal mucous membrane may be produced by the application of guinea pig serum to the nose. Nasal symptoms produced by a subsequent application of serum to the nasal mucosa closely resemble the symptoms of hay fever. The experiments were performed on naturally hypersensitive human beings (atopic individuals).

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THE ULTRAFILTRATION OF RAGWEED POLLEN EXTRACTS*

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THE chemical nature of the hay fever producing substance in ragweed pollen has been the subject of extensive investigation. It has been regarded as a protein,[1] a nonprotein,[2] and a complex carbohydrate.[3] The main methods used for the separation of this active substance from the crude pollen extracts are those designed to throw hydrophilic colloids out of solution. Thus most workers have used ammonium sulphate or high concentrations of alcohol for this purpose.

In the present study, it was planned to avoid, as far as possible, chemical treatments in the attempts at isolating the active allergen by utilizing other methods of separating colloidal from noncolloidal substances. Chief among the nonchemical procedures are dialysis and ultrafiltration. The latter, which is a modification of the former,[4] was chosen for this investigation since it offers several advantages: (1) the entire filtrate (dialysate) is recovered undiluted in the same volume as in the original solution; (2) the residue (dialyzed material) is obtained in a concentrated state; (3) the procedure is rapid as compared with the usual methods of dialysis.

Ultrafiltration consists in forcing a solution through a semipermeable membrane by means of pressure. The membrane retains the colloidal particles and thus removes them from the solution while allowing the substances in true solution to pass through. The filter† as used in our work consists of a short brass cylinder three inches long with an internal diameter of two inches, its walls being about three-eighths of an inch in thickness. At each end there is a wide flange extending laterally which provides a means for bolting into place a top and a bottom disc of heavy brass, thus converting the cylinder into a chamber. Rubber washers are used between the joints. An air-valve in the upper disc is connected by means of heavy rubber tubing to the source of pressure, which in this case, is a tank of compressed nitrogen. The lower disc has a short brass outlet tube. All the metal parts of the filter are silver plated. For filtration there is used a rubber washer, then a disc of cellophane supported by hardened filter paper, and a disc of brass

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†We are indebted to Mr. Lorenz Westvig, chief engineer at the Post-Graduate Hospital, for his interest and cooperation in making this filter.

Read before the Tenth Annual Meeting of the Society for the Study of Asthma and Allied Conditions Atlantic City, N. J., April 28, 1934.

gauze, placed at the lower end of the cylinder. The bottom disc is then bolted securely in place. The sample to be filtered having been placed in the cylinder, the top piece is bolted on and the pressure applied. A reducing valve permits a maximum of 70 pounds per square inch, or 4.7 atmospheres, to be obtained, a pressure at which three or four cubic centimeters of water will filter in an hour through a thin (No. 300) cellophane membrane. This maximum pressure was used in the following experiments.

FILTRATION

The extracts used in this study were prepared from various samples of high and low ragweed pollen, not more than three years old, which had been thoroughly dried, ether defatted, and stored in air-tight, moisture-free containers. Three grams of each pollen were extracted in 100 c.c. of alkaline saline solution[5] for three hours. The resulting extract, after being filtered through paper, was sterilized by passing it through a Seitz or Berkefeld filter.

In the first experiment 53 c.c. of ragweed pollen extract were ultrafiltered at room temperature through a thin (No. 300) cellophane membrane, a grade which had proved successful in retaining blood proteins. An interval of twenty hours was necessary for complete filtration. The dry dark brown residue on the filter was immediately taken up in the original volume (53 c.c.) of buffered saline solution[5] thus bringing the active substance of the residue to the same concentration it had in the original extract. The filtrate (No. 9F) and the residue (No. 9R) were then sterilized by filtration through Seitz filters. The protein and non-protein nitrogen content was determined on each and on the original extract by precipitation with phosphotungstic acid according to the method of Stull, Cooke and Tennant.[6] The three solutions were then diluted with the same volumes of sterile buffered saline solution that were used to dilute the original extract to concentrations of 0.01, 0.001 and 0.0001 mg. of total nitrogen per cubic centimeter. Tests were then made for allergen content by the direct intracutaneous method upon ragweed sensitive individuals. This same procedure was followed in all the subsequent experiments, unless otherwise noted, except for the last three experiments where indirect testing was also employed. In this experiment both the filtrate and the residue had approximately the same degree of activity. In other words, a considerable amount of activity passed through this thin cellophane membrane.

In a second experiment, a repetition of the first, 156 c.c. of the same extract were passed through thin (No. 300) cellophane, requiring a period of sixty hours. After the filtrate (No. 25F) was collected, the residue on the filter was washed by passing three volumes, 50 c.c. each, of buffered saline through the filter. This was done in an attempt to remove more activity from the residue. The washings (No. 25W) were

combined, and the residue, which finally remained after this procedure, was then extracted in 156 c.c. of buffered saline (No. 25R). The three fractions, residue (No. 25R), combined washings (No. 25W), and filtrate (No. 25F), were then compared for activity by intracutaneous tests after the proper dilutions had been made. The activity of the residue and the filtrate fractions was comparable as in the case of the first experiment, while that of the washings was slight.

In a third experiment 60 c.c of a fresh extract of ragweed pollen (No. 35) were prepared as above but with extra washings of the pollen with alcohol to remove gummy substances as recommended by Stull, Cooke and Chobot.[7] This extract was filtered through thin (No. 300) cellophane, an interval of twenty hours being required. The activity again was found to have been divided between the residue (No. 36R) and the filtrate (No. 36F), the latter, however, possessing the smaller degree of potency (Table I).

The results of these three experiments suggest that the allergen of ragweed pollen is of such a size that it is able to pass through No. 300 cellophane in considerable degree. This finding is in accord with other workers who have noted that the active pollen substances will dialyze partially.[8] In the attempt to find a membrane which would be dense enough to retain the allergen, the heavy (No. 1200) cellophane recommended by Coca[9] for dialysis was selected. Fifty c.c. of the same extract (No. 35) were filtered, approximately seventy-two hours at room temperature being required to complete the filtration in contrast to the twenty hours where a thin (No. 300) cellophane membrane had been used. The residue on the filter was dried overnight at 55° C., and then, cellophane and all, was ground in a mortar and extracted with 50 c.c. of buffered saline solution. Dilutions were made and examined by intracutaneous test. Practically all of the activity was retained with the residue, very little passing through with the filtrate (Table I).*

In the fifth experiment an attempt was made to separate the allergen from both larger and smaller particles by passing it through a series of filters of increasing density. A relatively porous membrane which allowed water to filter rapidly, about 60 c.c. an hour, was prepared by soaking a sheet of hardened filter paper (Whatman No. 50) for several days in a 3 per cent solution of collodion in glacial acetic acid. The membrane was allowed to drain while being rotated slowly so as to cause the collodion to spread evenly. It was then immersed in distilled water overnight. Fifty c.c. of a fresh extract (No. 44), prepared in the same manner as No. 35, were filtered in one hour through the very porous 3 per cent collodion membrane so prepared. The residue (No. 45A) was immediately taken up in 50 c.c. of buffered saline solution. The filtrate,

*This filtration method of concentrating the allergen suggests the possibility that it could be dried and stored on the filter membranes until needed for use. This is being investigated by us since Rappaport[10] has demonstrated the possibility of preserving the active substance in a dry state.

TABLE I

COMPARATIVE CUTANEOUS ACTIVITY OF RESIDUES AND ULTRAFILTRATES

CASE NO.	SAMPLE AND FILTER	DILUTIONS USED IN TESTS MG. N PER C.C.			
		0.0001	0.001	0.01	Undiluted
1	Original extract (control) 35	+++	++++		
	# 300 cellophane residue 36 R	++	+++		
	# 300 cellophane filtrate 36 F	+	++		
5	Original extract (control) 35	+++	+++	++++	
	# 300 cellophane residue 36 R	+++	+++	+++	
	# 300 cellophane filtrate 36 F	++	++	+++	
7	Original extract (control) 35	++	+++	++++	
	# 300 cellophane residue 36 R	++	+++	+++	
	# 300 cellophane filtrate 36 F	++	++	++	
12	Original extract (control) 35	++	+++	+++	
	#1200 cellophane residue 38 R	++	+++	+++	
	#1200 cellophane filtrate 38 F	+	+	+	
13	Original extract (control) 35	++	+++	+++	
	#1200 cellophane residue 38 R	++	++	+++	
	#1200 cellophane filtrate 38 F	0	0	+	
14	Original extract (control) 35	+	++	+++	
	#1200 cellophane residue 38 R	+	++	+++	
	#1200 cellophane filtrate 38 F	0	0	+	
16	Original extract (control) 50	++	+++	++++	
	#1200 cellophane residue 51 R	++	+++	++++	
	#1200 cellophane filtrate 51 F	0	0	0	+ (0.15)
17	Original extract (control) 50	++	+++	++++	
	#1200 cellophane residue 51 R	++	+++	++++	
	#1200 cellophane filtrate 51 F	0	0	0	+ (0.15)
19	Original extract (control) 50	++	+++	++++	
	#1200 cellophane residue 51 R	++	+++	++++	
	#1200 cellophane filtrate 51 F	0	0	0	++ (0.15)
21	Original extract (control) 52	++	++	+++	
	#1200 cellophane residue 53 R	++	++	+++	
	#1200 cellophane filtrate 53 F	0	0	0	+ (0.29)
22	Original extract (control) 52	+++	+++	++++	
	#1200 cellophane residue 53 R	+++	+++	++++	
	#1200 cellophane filtrate 53 F	0	0	0	0 (0.29)
24	Original extract (control) 52	++	+++	++++	
	#1200 cellophane residue 53 R	++	+++	++++	
	#1200 cellophane filtrate 53 F	0	0	0	+ (0.29)
27	Original extract (control) 54	+	+++	+++	
	#1200 cellophane residue 55 R	+	++	+++	
	#1200 cellophane filtrate 55 F	+	+	+	++ (0.40)
28	Original extract (control) 54	+	++	+++	
	#1200 cellophane residue 55 R	++	++	+++	
	#1200 cellophane filtrate 55 F	+	+	+	+ (0.40)
31	Original extract (control) 54	+	++	+++	
	#1200 cellophane residue 55 R	++	++	+++	
	#1200 cellophane filtrate 55 F	0	0	0	+ (0.40)

without further treatment, was then passed through thin (No. 300) cellophane, an interval of twenty hours being required. The second residue, collected on this, was taken up in 50 c.c. of buffered saline (No. 45B). The second filtrate was then passed through heavy (No. 1,200) cellophane, an interval of seventy-two hours being required. The residue (No. 45C) was taken up in 50 c.c. of buffered saline. The final filtrate was designated as No. 45D. After redissolving the three residues, the colors varied from light yellow in No. 45A, to a darker yellow in No. 45B, and to brown in No. 45C. On testing for allergen content, positive reactions were obtained with the residue solutions, these being quite comparable with the original extract, though of somewhat less intensity (Table II). The test with the final filtrate (No. 45D) was abruptly negative indicating that the activity contained in the previous residues had been removed by the heavy cellophane. It is well to remember, in this series, that the sum of the nitrogen contents and of the activities of the fractions B, C, and D represents that of the filtrate of A, while the sum of C and D represents the filtrate of B.

TABLE II

COMPARATIVE ACTIVITY OF VARIOUS FRACTIONS OBTAINED BY SUCCESSIVE ULTRAFILTRATION

CASE NO.	DILUTIONS MG. N PER C.C.	CONTROL	FRACTIONS USED IN TESTS			0.21
			RESIDUE 3% COLLOD.	RESIDUE #300 CELL.	RESIDUE #1200 CELL.	FINAL FILTRATE
		#44	45A	45B	45C	45D
32	0.0001	++	++	++	++	0
	0.001	+++	+++	+++	+++	0
	0.01	++++	+++	+++	+	0
33	0.0001	+++	+++	+++	++	0
	0.001		+++	+++	++	±
	0.01	++++	++++	+++	++	±
34	0.0001	++	++	++	++	±
	0.001	+++	+++	+++	+++	±
35	0.0001	+++	++	++	++	±
	0.001	+++	++	++	++	±
	0.01	++++	+++	+++	++	±
36	0.01	++++	++++	++++	++++	++
37	0.01	++++	++++	++++	++++	++

Extract No. 50 was prepared in the same manner as extracts No. 35 and No. 44, from low ragweed pollen collected in 1932. It was ultrafiltered through No. 1,200 cellophane, the residue on the filter being made as No. 51R and the filtrate as No. 51F. Due to the lack of activity in the previous ultrafiltrates when diluted, in this and the next two experiments, the ultrafiltrates were tested also in full strength. Some slight activity was found in the filtrates by this method, but in most cases it was negligible (Table I). In no instance was the activity of the filtrate, even in concentrated solution, more than that demonstrated in the 0.001 dilution of the control.

Extract No. 52 was similar to No. 50 but prepared from 1932 high ragweed pollen. It was ultrafiltered through No. 1,200 cellophane, and the residue and filtrate were designated No. 53R and No. 53F respectively. Here again, as in the preceding experiments, the residue was shown to contain practically all of the activity. In this case and the one following there was some trouble in maintaining the pressure so that the filter was opened before the filtration was quite complete. In these two cases, therefore, nonprotein nitrogen was found in the substance which was retained on the filter (Table I).

Extract No. 54 had been prepared the year previously for use in the clinic, having been made from pollen defatted with ether but not washed with alcohol, in this respect differing from the extracts except for No. 9. Both of these extracts had higher nonprotein nitrogen content, a higher phosphotungstic acid precipitable nitrogen content in the filtrate, and slightly greater activity in the filtrate than the extracts prepared from alcohol washed pollen. Again in this case, although to a slightly greater extent, only traces of the active material went through the heavy (No. 1,200) cellophane membrane during the ultrafiltration as shown by the tests with the undiluted ultrafiltrate (Table I). A small amount (7 per cent) of nonprotein nitrogen was found in the material on the filter as is explained in the previous experiment.

NITROGEN DETERMINATIONS

It seems evident from these experiments that the active substance in the pollen can be retained by sufficiently dense membranes. The ultrafiltrate, which corresponds to the dialysate in the usual dialyzing procedures, contains very little of the active substance. These inactive ultrafiltrates, however, do contain practically all of the nonprotein nitrogen of the original extracts while the active residues apparently have none (Tables III and IV). This supports the idea of Cooke and Stull[1] that nitrogen which is not precipitable by phosphotungstic acid is not part of the allergenic substance and therefore not to be considered in the determination of pollen activity. On the other hand the precipitable nitrogen, considered by them as a more accurate index of pollen activity, was not retained entirely in the active residue even by a thick membrane, approximately 20 per cent to 30 per cent passing through into the filtrate unaccompanied by a proportionate or even an appreciable amount of activity. The loss of such a considerable portion of the phosphotungstic acid precipitable nitrogen is difficult to understand in view of the fact that such nitrogen is ordinarily considered to be protein. It may be that this filtrable fraction, representing 7 per cent to 15 per cent of the total nitrogen content, is composed of simple polypeptides which are noncolloidal and therefore pass through the membrane readily, an explanation which was offered by Coca[11] for the presence of this fraction in his

TABLE III

NITROGEN DETERMINATIONS ON THE ULTRAFILTERED EXTRACTS

SAMPLE AND FILTER	COMPARATIVE INTRA-CUTANEOUS ACTIVITY	NONPROTEIN		P. T. A. PRECIPITATE		TOTAL	
		MG. N PER C.C.	PER CENT	MG. N PER C.C.	PER CENT	MG. N PER C.C.	PER CENT
# 9 original extract (not alcohol washed)	++++	0.204	100	0.146	100	0.350	100
# 9R # 300 cellophane residue	++	0.000	0	0.000	0	0.000	0
# 9F # 300 cellophane filtrate	++	0.185	91	0.064	44	0.249	71
#35 original extract	++++	0.161	100	0.155	100	0.316	100
#36R # 300 cellophane residue	+++	0.000	0	0.031	20	0.031	10
#36F # 300 cellophane filtrate	+	0.185	115	0.049	32	0.234	74
#35 original extract	++++	0.161	100	0.155	100	0.316	100
#38R #1200 cellophane residue	++++	0.000	0	0.070	45	0.070	22
#38F #1200 cellophane filtrate	+	0.160	99	0.027	17	0.187	59
#50 original extract	++++	0.088	100	0.067	100	0.155	100
#51R #1200 celophane residue	++++	0.000	0	0.024	36	0.024	15
#51F #1200 cellophane filtrate	+	0.087	99	0.021	31	0.108	70
#52 original extract	++++	0.141	100	0.151	100	0.292	100
#53R #1200 cellophane residue	++++	0.025	18	0.059	39	0.084	29
#53F #1200 cellophane filtrate	+	0.125	89	0.017	11	0.142	49
#54 original extract (not alcohol washed)	++++	0.280	100	0.123	100	0.403	100
#55R #1200 cellophane residue	++++	0.019	7	0.073	59	0.092	23
#55F #1200 cellophane filtrate	+	0.221	79	0.041	33	0.262	65

TABLE IV

NITROGEN DISTRIBUTION ON SUCCESSIVE ULTRAFILTRATION OF A RAGWEED POLLEN EXTRACT

SAMPLE AND FILTER	COMPARATIVE INTRACUTANEOUS ACTIVITY	NONPROTEIN		P. T. A. PRECIPITATE (PROTEIN)		TOTAL	
		MG. N PER C.C.	PER CENT	MG. N PER C.C.	PER CENT	MG. N PER C.C.	PER CENT
#44 original extract	++++	0.127	100	0.095	100	0.222	100
#45A 3% collodion residue	+++	--		--		0.020	9
#45B # 300 cellophane residue	+++	--		--		0.020	9
#45C #1200 cellophane residue	+++	--		--		0.022	10
#45D #1200 cellophane final filtrate	slt.	0.122	96	0.019	20	0.141	63

dialysates. The determination of the phosphotungstic acid nitrogen would therefore, from these findings, appear not to be an altogether accurate method of estimating the active substance. It would seem decidedly more logical, however, than the total nitrogen determination which includes definitely inactive and noncolloidal material.

It will be noted that in the first experiment there was obtained an active residue (9R) which apparently contained no nitrogen. It has been impossible to repeat this result or to explain it satisfactorily. It was due presumably to an error in performing the experiment.

In the passing of extract No. 44 through a series of filters of increasing density, the nonprotein nitrogen went completely through all the filters while the remaining nitrogen, assumed from our other experiments to be phosphotungstic acid precipitable, was approximately equally divided among the fractions, both final filtrate and residues, with a small amount lost in the process of reextraction (Table IV). If the phosphotungstic acid precipitable nitrogen was identified exclusively with the active substance, we should expect the final filtrate to be as potent as the residues; whereas, on the contrary, it was found to contain only faint traces of activity.

This observation furnishes an example of the inaccuracy of the intracutaneous test as an exact quantitative measure of allergic activity, a point which has been emphasized by Stull, Cooke and Chobot.[7] Obviously the allergen of the original extract was divided into three portions with a fraction absorbed to or trapped in the smaller pores of each filter membrane. However, in practically all of the comparative cutaneous tests the activity of the wheals obtained with the graduated dilutions of the extracts No. 45A, No. 45B, and No. 45C was similar to that from the corresponding dilutions of the original undivided extract No. 44, though slightly less pronounced. The comparative potency of extracts cannot be determined with mathematical certainty by the demonstration of wheals of the same size and activity.

DISCUSSION

The active product obtained by ultrafiltration is, of course, not a pure substance. It gives a strong Molisch test for carbohydrates but no biuret test for proteins. This is no proof that proteins are absent, for Stull, Cooke, and Chobot[7] report that their active material, when unpurified, did not give a biuret test but did so after the removal of excess carbohydrate.

While no conclusion as to the chemical nature of the allergen can be derived from this study, there is no doubt but that the active substance is colloidal. The reagents used by other investigators in its isolation are those which precipitate hydrophilic colloids. Furthermore, a sufficiently dense membrane will separate it from the noncolloidal substances

as shown by the fact that all the nonprotein (noncolloidal) nitrogen could be filtered off without removing any considerable amount of the active material.

CONCLUSIONS

The hay fever producing substance can be removed from solution by ultrafiltration through very dense membranes, such as No. 1,200 cellophane.

In like manner it is possible to remove completely the nonprotein nitrogen from ragweed pollen extracts without appreciably diminishing their activity.

Using this method, phosphotungstic acid precipitable nitrogen does not seem to correspond exactly with the allergic activity; though, at present, its quantitative determination affords the most practical chemical method of standardization of ragweed pollen extracts.

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DISCUSSION

DR. ROBERT A. COOKE, New York City.—The object, of course, of all of these studies of this type on pollen extracts is either to really determine the actual chemical nature of the active substance, or to find some method of improving and standardizing pollen extracts.

I have had the opportunity of reading and studying this paper which Dr. Newell has presented, and it seems to me that there is no question but that Dr. Spain and Dr. Newell have shown conclusively that the nonprotein nitrogen is not a factor concerned in the active substance; therefore, the method of standardization by total nitrogen is open to considerable objection.

As a corollary to this, they have definitely shown that the active substance itself is of a colloid type, without as yet going further into the question of demonstration of the chemical nature of the excitant.

Another interesting point that it seems to me is worth discussing here, is the fact that they have shown in these filtrates obtained by this method of theirs, that there is an appreciable precipitate in the relatively inert filtrate of substance precipitated by phosphotungstic acid. Undoubtedly some of this is colloid nitrogen (protein) as there is some activity in the filtrate, but certainly not enough to account for all this precipitable nitrogen.

In the work with Stull at our clinic published last year, we decided on phosphotungstic acid as it precipitated all the protein from solutions of pure pollen protein, whereas other precipitants, as sulphosalicylic acid, zinc sulphate, and others, did not always do so. We were aware that there is no chemical which on a single precipitation can show a sharp line of cleavage between protein and certain of its split amino products. Granted that there is an error of 20 per cent, the question is whether or not the phosphotungstic acid precipitable nitrogen of whole extract is a practical index of the activity of the whole pollen extract. We are pleased that the authors of the paper concur on this point. The answer to this, it seems to me, is to be found in the clinical test, and a severe test has been applied in the work from our clinic reported by Dr. Hebald this morning.

I must congratulate Dr. Spain and Dr. Newell on this work, which puts us another step in advance by establishing the inactivity of the noncolloidal nitrogen and confirming the colloidal nature of the active substance.

THE PRODUCTION IN THE RABBIT OF HYPERSENSITIVE REACTIONS TO LENS, RABBIT MUSCLE AND LOW RAGWEED EXTRACTS BY THE ACTION OF STAPHYLOCOCCUS TOXIN*

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IN A PREVIOUS report[1] it was shown that the intracutaneous injection of staphylococcus toxin produces an active immunity to multiple lethal doses of toxin in rabbits. In addition to this immunity the rabbit develops a hypersensitive state to the broth in which the toxin is produced. The skin reactions are as follows. The first injection of the toxin causes an area of erythema and swelling about 5 cm. by 5 cm. Necrosis occurs in some rabbits. Subsequent injections at intervals of one week cause lesser reactions; until after the fourth or fifth injections little or no reaction occurs. If the injections are continued there is a sudden increase in the size of the reaction. Injections of plain broth at this time cause a skin reaction equal to the one produced by the toxin, but at this time the rabbit is immune to the toxin. From this result it was concluded that the animal had developed a hypersensitive reaction to the broth. This sensitivity was explained by assuming that the growth activities of the staphylococci had separated from the medium some substance which, when attached to the toxin, became antigenic. In brief, a broth haptene was attached to the toxin. If this hypothesis is true, it is probable that other substances with little or no antigenic activity would become antigenic when combined with or attached to the toxin radical. It is the purpose of this report to show that rabbits can thus be sensitized or show an altered immune response to substances that are, ordinarily, poor antigens. The possible relationship of these findings to so-called bacterial allergy is discussed.

The first material used to test this hypothesis was lens substance because of the possible relationship of this substance to endophthalmitis phaco-anaphylactica. In 1922, Verhoeff and Lemoine[2] proposed this name for a certain type of postoperative intraocular inflammation following cataract extraction which they thought was due to a reaction between retained lens substance and ocular tissues hypersensitive to lens substance. This concept was based on the fact that many cataractous individuals with postoperative inflammation were sensitive

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Read before the Tenth Annual Meeting of the Society for the Study of Asthma and Allied Conditions, Atlantic City, N. J., April 28, 1934.

to lens when tested by intracutaneous injection of lens extracts, either of human or of animal origin. Normal individuals tested with the same extracts were not skin reactive. They also found a characteristic histologic picture in the eyes removed after the development of such inflammation. In these eyes the lenses were invaded by leucocytes and macrophages, and there were no demonstrable bacteria.

This hypothesis of the allergic etiology of endophthalmitis phacoanaphylactica has had a considerable following, but its universal acceptance has been prevented largely by the failure of attempts to reproduce the disease experimentally. A recent communication from Dr. Verhoeff states, "We entirely failed to reproduce the phacoanaphylactic reaction in rabbits." Since 1922 various reports have appeared, but to our knowledge not one of them has confirmed the hypothesis experimentally. Braun,[3] in particular, has denied its truth because he was unable to produce precipitins for or to sensitize the animals to lens substance. Since Uhlenhuth's[4] original announcement of the organ specificity of lens protein, proved by the production of precipitins for this substance, numerous confirming reports of this fact have appeared. However, few workers have reported the development of anaphylactic or hypersensitive states in the rabbit, and an analysis of such reports leaves one in doubt whether the deaths were anaphylactic or anaphylactoid. An acute anaphylactic death in the rabbit is extremely difficult to obtain even with the most active antigens. Our personal observations, extending over a period of eight years, indicate that it is extremely difficult, if at all possible, to develop an anaphylactic state in the rabbit with lens extracts. To our knowledge no one has reported the development of an Arthus reaction to lens substance in the rabbit. With the hope of establishing such a state the following experiments were undertaken:

Beef eyes, trimmed of all extraocular tissue, were dropped into boiling water for thirty seconds. With a sterile scissors a transverse corneal incision was made and the lens expressed into a tube containing 20 c.c. of hormone bouillon. After a preliminary incubation of seven days to eliminate the contaminated tubes, the sterile tubes were inoculated with toxin-forming *Staphylococcus aureus*, strain Ha. After ten days' incubation at 37° C. the contents of the tubes were pooled, 0.5 per cent trikresol added and filtered through a Berkefeld V filter. Injected intravenously, 0.3 c.c. killed a 3,000 gm. rabbit within twenty-four hours. The lens broth toxin precipitated with lens immune serum in dilutions as high as 1:50,000.

Nine rabbits were injected intracutaneously with lens broth toxin and lens extract. Each rabbit received 0.1 c.c. of each preparation at intervals of one week. Two rabbits were injected intravenously, once a week, with 5 c.c. amounts of lens extracts. Four rabbits were

injected intracutaneously with lens extract, beef serum, and mixtures of these two substances. Lens extract was prepared by emulsifying fresh lenses in N/100 ammonium hydroxide containing 0.5 per cent trikresol. After filtration through a Berkefeld V filter it was adjusted to contain 2 per cent protein by weight. For all other details of technic see References 1 and 5.

The initial injections of lens extracts did not produce in any of the rabbits any skin reactions except transient areas of swelling. The initial injections of lens broth toxin caused reactions like those produced by plain broth toxin. After the fourth or fifth coincident injections of lens broth toxin and lens extract, the latter substance produced areas of swelling with occasional necrosis in 8 of the 9 rabbits. The size of these reactions exceeded 2 cm. by 2 cm. and in many instances were as large as 5 cm. by 5 cm. The reactions appeared within four hours and persisted as long as forty-eight hours. At this time extracts of human, swine and rabbit lens produced similar reactions. Such reactions to lens extracts were not seen in the other experimental animals or in normal controls.

Lens precipitins were not present in any of the rabbits before injections were begun. The highest precipitin titers were found in the animals injected intracutaneously with lens broth toxin and lens extract. These titers reached 1:50,000 or more after the injection of about 1 c.c. Those injected intravenously had titers of about 1:5,000 after the injection of about 25 c.c. Lens precipitins were not found in the animals injected with lens extract and beef serum.

The above mentioned experimental animals and 4 normal controls were subjected to a modified needling operation after the skin sensitivity had appeared. Under cocaine anesthesia, a 25 G. needle attached to a syringe was inserted into the anterior chamber and about 0.2 c.c. of aqueous humor was drawn into the syringe. The needle was then thrust into the lens and the aqueous injected.

The skin sensitive animals developed marked intraocular inflammation characterized by conjunctival and pericorneal injection, steamy cornea with vascularization, positive aqueous ray and plastic iritis. The ocular inflammation continued for at least seven days in the eyes not removed for histologic study before this time. The immune but insensitive animals reacted like the normal controls with a purely traumatic reaction which subsided in less than seven days. The experimental results are summarized in Table I.

Subsequent histologic examination of the needled eyes confirmed the clinical findings except in one instance. Rabbit 13, diagnosed clinically as "infected," showed the histologic picture of endophthalmitis phaco-anaphylactica. From this eye was isolated a toxin-forming staphylococcus. It is possible that this organism, acting on the

TABLE I

RABBIT NO.	LENS NEEDLED AFTER .... INJECTIONS	SKIN SENSITIVITY TO LENS EXTRACT AT TIME OF NEEDLING	OCULAR REACTION		DAY OF ENUCLEATION	PRECIPITIN TITER AT TIME OF NEEDLING
			CLINICAL[1]	HISTOLOGIC		
		GROUP I				
		RABBITS IN THIS GROUP INJECTED WITH LENS BROTH TOXIN AND LENS EXTRACT, INTRACUTANEOUSLY				
1	5	Waning[2]	Positive	Doubtful pos.	15	50,000
2	5	Waning	Positive	Doubtful pos.	15	50,000
3	4	Positive	Positive	Not removed		50,000
4	4	Positive	Positive	Not removed		50,000
5	4	Positive	Positive	Positive	15	50,000
6	4	Positive	Positive	Hemorrhage[3]	2	50,000
7	4	Positive	Positive	Positive	15	50,000
8	4	Positive	Positive	Positive	8	50,000
9	4	Negative	Trauma	Negative	15	50,000
		GROUP II				
		RABBITS IN THIS GROUP INJECTED WITH LENS EXTRACT, INTRAVENOUSLY				
10	4	Negative	Trauma	Negative	15	5,000
11	4	Negative	Trauma	Not removed		5,000
		GROUP III				
		NORMAL RABBITS				
12	0	Not tested	Trauma	Negative	2	0
13	0	Not tested	Infected[4]	Positive[5]	15	0
14	0	Not tested	Infected[4]	Negative	15	0
15	0	Not tested	Trauma	Negative	15	0

[1]Positive-phaco-anaphylactica, as shown by palpebral and conjunctival injection, pericorneal injection, scleral necrosis, corneal haze with late vascularization, aqueous ray, iritis, synechia and lens opacities.

[2]Waning—Previous positive skin reaction to lens extract but only weakly positive at time of needling.

[3]This rabbit showed the most intense reaction after needling. Within forty-eight hours there was a necrotic rim about the cornea 2 mm. wide. It was thought that the eye would rupture and enucleation was attempted. The eye ruptured while it was being removed. The entire picture, histologically, was obscured by hemorrhage throughout the eye. Bacteria were not found.

[4]The clinical picture in these eyes was much like the one described in f. n. 1. In addition there was hypopyon and a purulent discharge. Cultures from the discharge showed toxin-forming Staph. aureus and a gram-negative rod thought to be the snuffles organism.

[5]See the discussion in the text.

damaged lens, actually sensitized the rabbit to lens and thus produced the histologic picture of endophthalmitis phaco-anaphylactica. To comment further, however, on this finding would require a discussion too long for the allotted time.

These results show clearly that rabbits can be made allergic to and develop precipitins for lens substance, one of their own tissues. This substance is, however, organ specific and has an antigenic activity which is greater than that of other tissues. To avoid this complicating factor, if possible, rabbit muscle was substituted because this substance is not known to contain an organ specific fraction. Rabbit muscle toxin was prepared by inoculating strain Ha to a hormone type medium made from rabbit muscle without the addition of any foreign substance except sodium chloride. A filtrate, prepared as described, was lethal for rabbits in doses of 0.1 c.c. per kilo. Six rabbits were injected intracutaneously at intervals of one week with the following results:

The initial injections produced areas of erythema and swelling as large at 10 cm. by 10 cm. in the majority of the rabbits. The swelling was marked, and after twenty-four hours was covered with a serous exudate. Necrosis did not occur. The reactions were almost identical with those produced by the intracutaneous injection of small numbers of live staphylococci. Subsequent injections produced lesser reactions; until about the fifth injection the reactions did not exceed 3 cm. by 3 cm. At this time rabbit muscle broth alone produced a similar reaction in four of the six rabbits. It produced no reaction in the two other rabbits in this group and none in eighteen other rabbits receiving a varied assortment of intracutaneous injections. Rabbit serum caused no reactions in any of these animals. These findings indicate that the rabbits had become reactive to some substance derived from rabbit muscle.

This group of rabbits showed other reactions which suggested that physical trauma was releasing from the skin or underlying musculature the substances to which they were skin reactive. For example, these animals were of the type that require repeated depilation for continued skin injections. Each depilation caused a severe dermatitis which did not occur in control animals. Pinching of the skin with a hemostat caused an area of erythema and swelling which was identical with that produced by the injection of rabbit muscle broth. Such a reaction was not found in control rabbits.

The reactions in the above rabbits following the later intracutaneous injections of rabbit muscle toxin were not due primarily to the toxin because when skin tested with beef broth toxin they were nonreactive. They were also immune to large intravenous doses of toxin and live cultures of staphylococci.

These results indicate that some rabbits can become skin sensitive to substances derived from their own tissues and not known to be organ specific. There is a strong suggestion that the reactivity of these animals to physical trauma has been altered and that the reactions following trauma are due to the liberation of substances from the animal's own tissues and to which the animal has been previously sensitized.

These results may have some bearing on disease pictures in human beings which are ordinarily ascribed to hypersensitive states of unknown etiology. Ophthalmologists are familiar with cases of recurrent iritis which are associated with so-called focal infection. In such cases it is customary to assume that at irregular intervals, showers of bacteria or their toxic products are liberated from a focus of infection and reach the previously sensitized eye with a resulting ocular inflammation. In many instances removal or treatment of the supposed focus alters the course of the iritis. In many of these cases, however, organisms cannot be recovered from the focus or the eye. These facts in themselves suggest that bacteria need not be present, and another point of view suggests itself. An infection of a focus with staphylococci or possibly other organisms sensitizes the individual to some element of the infected tissue, just as the rabbits were sensitized to lens and muscle extracts. At some future time a reinfection or other trauma of the focus liberates the sensitizing substance which causes the ocular inflammation. This hypothesis does not involve any radical departure from previously accepted ideas but merely shifts the emphasis now placed on bacterial allergy to an autogenous tissue sensitivity brought about by the action of bacteria. The practical suggestion arising from this point of view is that patients of this type should be tested for "self-sensitization" rather than with a variety of bacteria.

If the rabbits sensitized to lens substance and rabbit muscle extracts had reached a clinician without any history of their previous treatment and with only a suspicion in his mind concerning their allergic state, skin tests with bacterial substances would only obscure the picture, for it must be remembered that rabbits sensitive to rabbit muscle do not react to beef broth toxin and animals sensitive to lens do not react to rabbit muscle toxin. Therefore, any of these rabbits tested with appropriate staphylococcus preparations would be nonreactive, while they might be highly reactive to any one of a number of other species of bacteria. From such results the conclusion would be that staphylococci played no rôle in the development of the allergic state.

Staphylococcus toxin has also been combined with low ragweed pollen extracts in the same manner as was lens substance. This rag-

weed toxin injected intracutaneously at intervals of one week produced precipitins for low ragweed extract. It also altered the rabbits' reactivity to low ragweed pollen. After twelve injections three rabbits were skin tested with low ragweed extract and found to be highly reactive. They were then placed in a pen with walls three feet high. Before dusting with the pollen they were perfectly placid. Shortly after the dusting there was some nose rubbing followed by an excited stage. The preliminary movements suggested the beginning of an anaphylactic state. There were tremors and short runs which ended by leaps into the air sufficiently high to clear the walls of the pen. Following the excitement stage two of the animals became cyanotic, and the depilated areas on the back were tinged with a deep blue. The conjunctivae were markedly congested, and a purulent secretion appeared within thirty minutes. Normal rabbits and rabbits injected with ragweed extract alone did not show any such signs after dusting with the pollen.

At this time it is not clear why staphylococcus toxin alters the antigenicity of ordinarily inert substances. However, the results reported here and other experiments which have been done all point to the fact that it has the ability to increase antigenicity. Somewhat similar observations have been made before, but this explanation has not been previously suggested. Panton and Valentine[6] injected whole cultures of staphylococci intracutaneously and found that after several injections an immune and an allergic state developed. The allergic state was ascribed to the bacteria, but the possibility of a media sensitivity has not been ruled out. Neill and his associates,[7] likewise, have reported a diphtheria toxin sensitivity, but their results are open to the same criticism. Hooker[8] has found that diphtheria toxin-antitoxin mixtures produce more marked horse serum sensitivity in human beings than does horse serum alone. Rosenau[8] has found that diphtheria toxin-antitoxin mixtures produce more marked anaphylactic reactions to horse serum in guinea pigs than does horse serum alone. These latter findings suggest that the ability to increase antigenic action of other substances may be inherent in other toxins.

SUMMARY

By combining staphylococcus toxin with lens substance rabbits have been sensitized to lens and have developed high precipitin titers for this substance. The clinical and histologic appearance of the eyes of such sensitive rabbits after needling duplicates the picture found in human cases of endophthalmitis phaco-anaphylactica, a disease suspected of being due to an allergic reaction to lens substance.

Staphylococcus toxin produced in rabbit muscle broth has sensitized the rabbit to the broth alone. Such rabbits show an altered reactivity

to trauma which may be due to the animal's reaction to substances in the tissues which are liberated by the trauma and which are also contained in the broth to which the animal is reactive.

Staphylococcus toxin combined with low ragweed extract produces precipitins for low ragweed extract in the rabbit. Dusting with low ragweed pollen causes anaphylactic-like symptoms in such rabbits.

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DISCUSSION

DR. ROBERT A. COOKE.—The paper that Dr. Burky has just presented for our consideration seems to open up so many new broad and inviting possibilities that it is fairly bewildering.

As I understand it, what Dr. Burky has discovered is a new way of increasing the antigenic capacity of certain proteins—possibly all proteins—by what may be called a synergistic or perhaps a catalytic action of something in staphylococcus toxin broth filtrates. Many attempts have been made to increase antibody response to antigen; e.g., formaldehyde added to toxin broth produces toxoid which enhances the antibody response; and recently Ramon and Lemetayer have found that tapioca or calcium chloride added to the toxin also increases the antibody output. But in Dr. Burky's study the antibody response is not to the toxin but to an associated protein and even homologous protein (muscle).

One of the observations that seems to me to be of the greatest significance is the secondary effects noted in rabbits sensitized to their own muscle; that is, the production of a sort of wheal on pinching and a dermatitis from the depilatory. This opens up the possibility that cases of dermographia and certain of the eczemas heretofore not understood may be on their way to a solution through studies which shift the emphasis, as Dr. Burky says, from the bacteria to the tissues.

Again I have been impressed by the ease with which he has produced reaction to ragweed pollen. In experiments in our laboratory even after 15 injections of 0.4 mg. pure protein we were not able to get a reaction by skin test and only a weak precipitin reaction in 1:500 dilution. This has been the experience of all workers using ragweed experimentally as antigen.

I agree with Dr. Burky that the described reaction to inhaled ragweed pollen in sensitized rabbits is nothing other than a typical anaphylaxis analogous to that reported by Osborne and Wells and others. This, of course, is not the immunologic picture of hay fever in human beings. In certain forms of allergy in man we are not yet sure that

contact with antigen is a necessary precursor to sensitization. From analogy with experimental allergy in animals we assume it to be so. But it does seem to be proved that heredity is an important predisposing cause. Dr. Burky reports that but four out of six rabbits treated with ragweed toxin broth became sensitized. It might be possible to breed rabbits so that 100 per cent would be sensitized or 100 per cent would be resistant, and hence an hereditary factor would be demonstrated for animals as it now seems to exist for man. Given, then, the proper infection plus the proper exposure to antigen, plus the hereditary background in man, and sensitization ensues. This hypothesis is interesting and warrants thought and observation.

I should like to know whether such rabbits would give a Dale reaction. I should also like to ask Dr. Burky whether he means that the skin reactions in rabbits so sensitized to ragweed were immediately positive as in our hay fever cases; that is, within fifteen or twenty minutes, or just what was the appearance time of the reaction. An eye test in these rabbits would also be interesting.

It is always difficult to evaluate the importance of results of studies as presented in this paper. I have the feeling that perhaps we have listened to a real epoch-making discovery in the field of immunology. I wish to thank Dr. Burky and to congratulate him upon his contribution to our program.

Dr. A. H. W. Caulfeild.—I should like to congratulate Dr. Burky on his paper and ask him some questions.

He states that he incubated the various extracts, or, as he called them, toxins, with a staphylococcus-toxin-producing culture. I should like to ask him whether instead of incubating with the staphylococcus-toxin-producing culture, he has tried or used just the staphylococcus toxin—in other words, did he need incubation with the staphylococcus culture rather than the toxin to get his results?

Another point which might interest Dr. Burky is the fact that staphylococcus toxin was first produced by Dr. Dolman, at the Connaught Laboratory, University of Toronto; and many of us there have had ample opportunity to observe the results of injection of staphylococcus toxoid among those infected by staphylococcus. It so happened that Dr. Dolman injected a patient suffering from boils, with his toxoid, and shortly after it she developed asthma, and he referred her to me. Her history was interesting. She had come from Europe some ten or fifteen years ago, and while there she had had a very severe case of asthma. Ever since coming to this country she had been entirely free from asthma. When she was injected with the staphylococcus toxoid, she rapidly developed an extreme degree of asthma, and in her own mind she was convinced it was the staphylococcus toxoid that was responsible. Dr. Dolman was convinced it could not have had anything to do with it.

As Dr. Cooke has said, this is very interesting as apparently presenting a means of developing sensitization very easily.

The Connaught Laboratories also produce various antiserums, and tapioca, which he mentioned, is then mixed with diphtheria toxin and other toxins, and is a very excellent way, they tell me, of inducing a high potency in the injected horses, but that is immunity and this which we have heard today is sensitization.

DR. EARL L. BURKY.—In answer to Dr. Cooke's first question about the Dale reaction, I have never used that test. In answer to the question whether the skin tests are delayed or immediate, when one starts the injections with the toxin, the reaction of the toxin is a delayed one. It sometimes does not appear for forty-eight hours; but as one continues the injections, the reactions appear in shorter time intervals. One of the ways of recognizing "by-product" sensitizations is that the reaction appears within four hours and persists for an indefinite period.

In answer to Dr. Caulfeild, if one injects toxin in one site and lens in another, sensitization to lens will develop in about one out of four animals. This method was used because we thought that we were possibly splitting up the lens by bacterial action and making some sort of by-product which was not essentially lens.

It is entirely possible that Dr. Caulfeild's patient got asthma from an injection of toxin. The toxin has peculiar reactions. One cannot always foretell what it is going to do in either human beings or rabbits. However, I should suspect that the asthma was due to some "by-product" that was brought into action by the toxin. I do not believe it was a real sensitivity to the toxin itself.

VASOMOTOR RHINITIS WITH NEGATIVE SKIN TESTS: A LOCAL NASAL ALLERGY*

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THE object of this paper is to present a classification of nasal allergy, a method of diagnosis heretofore not used routinely in vasomotor rhinitis with negative skin tests and a preliminary report of the results of treatment in such patients.

Much has been written in recent years on the subject of vasomotor rhinitis. The importance of protein hypersensitivity, bacterial sensitivity, endocrine dysfunction, neuroses and anatomic nasal abnormalities as etiologic and predisposing causes has been discussed and treatment directed at all and each of these factors has been advocated. All workers agree on the difficulties of diagnosis and the frequent unsatisfactory results of treatment. The number of etiologic factors and the numerous types of treatment are an indication of the difficulties of diagnosis, and the usually poor results of therapy.

Clinically nasal allergy may be divided into three groups as follows: (1) seasonal allergic rhinitis; (2) perennial allergic rhinitis with positive skin tests; (3) perennial allergic rhinitis with negative skin tests (vasomotor rhinitis).

The first two groups will be discussed briefly in order to define more accurately the third group.

In the first group or seasonal allergic rhinitis, symptoms are caused by the inhalation of pollen, and are limited to the seasons of pollination of the plant to which the patient is sensitive. Closely related to it is the symptom-complex known as perennial allergic rhinitis. Patients suffering from this symptom-complex show all the phenomena common to the seasonal variety, but symptoms occur throughout the year either constantly or in repeated attacks and are caused by sensitivity to a variety of inhaled or ingested substances to which positive skin tests are obtained in all cases.

The third group or perennial allergic rhinitis with negative skin tests is differentiated from the second group by certain definite immunologic characteristics. Whereas the patients belonging to the first two groups give very definite evidence of a familial tendency in a very large percentage of the cases and usually have other manifesta-

*From the Asthma and Hay Fever Clinic of the Asthma, Hay Fever and Allergy Foundation, Cleveland, Ohio.

Read before the Tenth Annual Meeting of the Society for the Study of Asthma and Allied Conditions, Atlantic City, N. J., April 28, 1934.

tions of allergy, such as asthma and eczema either concomitantly with the nasal symptoms or in the past history, those patients belonging to the third group usually present the nasal symptoms alone.

On examination the nose reveals a boggy edematous succulent mucosa and contains a watery discharge which on occasions may become semipurulent. There is often polypoid degeneration in the region of the middle turbinates. The surface is sometimes red and granular, but more often glistening, bluish gray or pink in appearance; both sides are involved although the condition is usually more marked on one side.

Transillumination of the sinuses often shows one antrum or the other cloudy, but following shrinking of the nasal mucosa the sinus usually becomes transluscent. On examination both by posterior rhinoscopy and also with the nasopharyngoscope small out pouchings of mucous membrane are frequently seen, especially in the region of the middle meatus. These little ballooned-out areas of the mucosa usually disappear after the attacks subside. (This in our opinion is the beginning of mucous nasal polyps.) Eosinophiles play an important part in the diagnosis. Often many slides will have to be taken to demonstrate the eosinophiles, since at times they are absent in allergic cases. The cells tend to be in groups, and the field on the slide examined may not be a true index of the total number present. Eyerman[1] reports that he has found eosinophiles reach as high as 72 per cent. Johnson and Goldstein[2] report from 5 per cent to 100 per cent eosinophiles present in their smears.

The indistinguishable clinical histories, physical and laboratory findings suggest that all cases of perennial rhinitis, whether with positive skin tests or without them are allergic, and that the differences which are more apparent than real might be explained if improved methods of study could be found.

In a consideration of 500 patients who had presented themselves with nasal symptoms 170 or 34 per cent had seasonal allergic rhinitis. Two hundred and ninety or 58 per cent had perennial allergic rhinitis, associated often with asthma, eczema or both, and who gave positive scratch or intradermal reactions.

Forty or 8 per cent had perennial allergic rhinitis without positive skin reactions. The first two groups gave a positive family history for allergy in 52 per cent of the cases, and 56 per cent had either asthma, eczema or urticaria associated with the nasal symptoms. In the third group 2 per cent gave a positive family history for allergy, and 10 per cent had asthma associated with the nasal symptoms.

It has always been interesting to note that the same substance produced different symptoms in different individuals. This was explained by Coca[3] on the theory of the shock organ, namely, that the

organs of the body varied in their degree of sensitivity, or in their actual sensitivity. Alexander[4] has also stated that "certain organs only are receptive to allergens, and that unless the skin is so, no reactions will occur. The skin will react in about 50 per cent of allergic patients."

It is not unusual to find patients with seasonal allergic rhinitis who give negative skin reactions. Nor is it less frequently found that patients will react to almost every allergen to which they are tested. Very often spurious reactions are encountered. The finding of patients with dermographism further complicates the picture. These experiences occur more often in the practice of allergy than are reported in the literature.

Because of the inadequacies of the scratch and intradermal methods a number of workers have resorted to other methods of testing.

Bernton,[5] Balyeat,[6] and Kahn[7] have advocated the hypodermic method. The ophthalmic conjunctiva has been used by Peshkin[8] and others. Up to the present, the nasal mucosa for testing has been used only in cases of pollinosis. Blackley[9] first suggested nasal mucous membrane testing, and later Dunbar[10] used it. Duke[11] recommends spraying pollen extracts into the nose in cases where it is desired to determine the causative pollen of a series producing positive skin reactions. In 1930 Efron and Penfound[12] reported on the systematic use of the nasal mucosa as a criterion in the diagnosis of seasonal hay fever. They used dry pollen sprayed into the nose. They give the following reason: "We feel that in all cases of pollinosis in which the nose is involved, the nasal mucosa is the final judge of the hypersensitivity, and that dry pollens used as allergens in the nose closely approximate conditions as they occur in nature."

Failure to determine causative factors in cases obviously allergic, led us to investigate these patients directly in the shock organ. Accordingly the nasal mucous membrane was tested in patients whom we had diagnosed as perennial allergic rhinitis with negative skin tests or "vasomotor rhinitis."

The twenty-two patients selected for this study had been under our care for from three months to two years, and the variations in their symptoms are well understood. Except for two patients each of whom gave a one-plus reaction to one substance, none other gave skin reactions, though most of these patients had skin tests repeated at least once. There were no clues in their clinical histories to assist us, and dietary experiments failed to give the hoped for relief. For all intents and purposes, these patients were failures from the standpoint of diagnosis and treatment.

THE TEST

The following materials and instruments were used for this test: dried epidermals of chicken, goose, duck, canary, cat, cattle, dog, horse, rabbit, and wool; cottonseed, flaxseed, kapok, mattress dust, orris root, pyrethrum, glue, grass and ragweed pollens. A 1 per cent aqueous ephedrine solution, normal saline, hospital cotton cut in pledgets one inch by two inches; smooth metal probes, a metal syringe, a nasal forceps and speculum. Each allergen is sprinkled on a pledget of cotton which is very slightly moistened with saline. This allows the material to adhere to the cotton. It is then placed on a metal probe and inserted against the mucous membrane of the middle turbinate and nasal septum. A control of normal saline is placed in the opposite nasal chamber. This is permitted to remain in the nose for at least twenty minutes. The cotton pledgets are then removed, and the patient is observed for another fifteen or twenty minutes. The test can only be performed when the patient has had at least a twenty-four-hour period of freedom from symptoms, and when on examination the typical reaction in the nose is absent.

When the material is placed against the mucous membrane, the immediate effects are produced in practically every instance whether the patient is hypersensitive or not.

There is some suffusion of the eyes and irritation of the nose which is present on both the tested and the control side. There may be burning, tingling and even pain over the bridge of the nose and the eyes, some formation of mucus and occasionally one or two sneezes. Examination of the nose at this time shows a very highly congested mucous membrane with little or no turgescence and a scant mucous discharge. If the patient is not hypersensitive to the particular inhalant used, these symptoms and signs will subside in several minutes and no further reaction will occur. When hypersensitiveness is present, signs of vasomotor rhinitis localized to the side tested will occur. Most patients will react within twenty minutes. However, one patient of our series reacted two hours later and another twelve hours after the test; therefore, it is not advisable to do more than one test in a twenty-four-hour period. In one of our patients edema of the soft palate and uvula was noted; in two others cough with wheezing was observed. No untoward effects were produced which could not be controlled by applications of tampons of 1 per cent ephedrine solution or irrigation with warm normal saline solution except in one patient who required adrenalin for the control of a generalized pruritis and nasal obstruction which was intractable for about one week.

In 19 of the 22 patients studied from one to four positive reactions were observed in each patient. The following is a typical case report:

H. K., a male thirty-five years of age, came to us for the first time in January, 1933, complaining of nasal obstruction and profuse nasal discharge which had been practically constant for two years. On this visit it was explained to him that unless we could have him under our care for a period long enough to make the necessary studies and give the proper treatment, we could offer him nothing in the way of any lasting relief. He did not return after this visit but returned five months later with a continuation of the same history, only in addition his return to us was motivated by fear of a radical operation on his nose which he was told he would have to undergo. On examination, we found a typical picture of perennial allergic rhinitis as described elsewhere in this paper. In addition, however, he did have a small ridge at the base of the septum on the left side. General examination revealed no findings of any significance. Scratch and intradermal tests gave us no clue. In one of his very brief free periods nasal tests were performed. He gave positive reactions to cotton and wool. These findings were of extreme interest to us in view of the fact that this man was in the dry goods business. Later he reported an acute attack of nasal obstruction with sneezing and discharge when he sorted some rag rugs. Treatment consisted of a series of inoculations alternating wool and cotton extracts. This was begun during the first week of July, 1933, and carried up quite rapidly with the result that since August there have been two twenty-four-hour periods of nasal difficulty and one seventy-two-hour period. Frequent examinations of the nose show a normal appearance of the mucous membrane without nasal obstruction and the absence of excessive nasal discharge. We feel, that removal of the septal ridge may give him a 100 per cent result.

Therapy has not continued long enough in all 19 patients who gave positive reactions to draw final conclusions as to the results of treatment. It has, however, been carried on sufficiently to show that eight more obtained relief varying in from 50 per cent to 90 per cent. The other 11 patients do not show sufficient change in their symptoms at present to enable us to draw any conclusions about them.

This work is being continued with additional studies using bacterial and mold filtrates.

SUMMARY AND CONCLUSION

1. The importance of a proper classification of nasal allergy has been emphasized in order that a proper statistical study of end-results may be obtained.

2. There is a group of cases of nasal allergy in which the reaction is confined to the nose. Skin tests are absent in this group. This group comprises 8 per cent in a series of 500 patients presenting themselves with symptoms of nasal allergy.

3. A method of nasal mucous membrane testing applicable primarily to this group but useful in other types of cases has been discussed and its limitations have been considered. The use of this test as a guide for treatment has resulted in marked improvement in over 40 per cent of 19 cases in which we had failed to obtain relief by other methods.

4. With greater experience in the use of this method the effect of substances other than the common inhalants are being studied, e.g., bacterial and mold filtrates and volatile substances.

5. Finally, this method is not to take the place of skin tests or other methods of diagnosis, but its use is suggested in all cases where skin tests are negative.

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DISCUSSION

DR. LOUIS TUFT, Philadelphia.—I am sure we will all agree with the authors that the problem of so-called vasomotor rhinitis, or, as I prefer to term it, perennial allergic coryza, offers similar difficulties to those encountered, for example, in urticaria.

I have always preferred to look upon this condition very much as asthma of the nose and to divide it, accordingly, into the sensitive and non-sensitive type, in accordance with Dr. Cooke's classification.

In reviewing the cases which I have seen, I find that in the sensitive group are patients whose symptoms are associated with other allergic manifestations, who have positive personal or family histories, and who give positive skin tests. On the other hand, in the second group this history is usually lacking and skin tests either are negative or at least very slight in degree.

I think we have here the same existing relationship in these two groups as occurs in serum sensitiveness, as Dr. Waldbott mentioned this morning. There is the natural or atopic type of serum sensitiveness in which the skin reaction is exceedingly marked, and, on the

other hand, there is the acquired type, in which skin sensitivity is likely to be greatly diminished or absent. Between the two groups there exists therefore a marked difference in the threshold of sensitivity.

Most of the patients in which Dr. Rudolph and Dr. Cohen report the use of the nasal test are contained in the second group. I believe that in many of these, food sensitivity has a more important relationship than we have been in the habit of ascribing to it; possibly one reason that we so frequently get negative, doubtful, or only slightly positive skin tests in this group is that there is a much lessened threshold of sensitivity. In my experience, many of these patients seem to do very well, after elimination of all the test-positive foods, even if they give doubtful reactions, or with the use of elimination diets.

Where food sensitivity exists, the type of test which Dr. Rudolph has described will, of course, have very little value, but should rather be used in the remaining types in which such factors cannot be found.

The only disadvantages I can see to the test are: first, the fact that it has to be carried out in a period of freedom from symptoms, which is not always easy to do; and second, that it takes a considerable length of time to carry out all the tests.

Dr. Salvatore J. Parlato, Buffalo.—I wish to congratulate both Dr. Rudolph and Dr. Cohen for their contribution. As a matter of fact, any study that can be offered to help us with these cases of vasomotor rhinitis is undoubtedly of great value.

Of course, we all have these cases which Dr. Rudolph classified as the typical perennial type of hay fever, but with negative skin tests. It is our common experience that in this type of case we have our greatest disappointments and failures.

In considering his test, there are several difficulties which have come to my mind, and Dr. Tuft referred to one of them, the necessity of finding a period of twenty-four hours where examination of the nose can tell that it is clear, and free from any pathologic manifestation. It has been my experience that in these cases, both the turgescence and the mucosal thickening, as well as the rhinorrhea, are more or less constant factors.

Another difficulty in this test is that it really requires the services of a nose and throat man, and we should all avail ourselves of a rhinologist's services in these special cases.

In Dr. Rudolph's paper he mentioned in this particular classification the presence of deflected septums and diseased sinuses. I think we have to consider some of the cases with negative skin tests as having positive sinus diseases. I wish to ask whether he uses cocaine and adrenalin in order to make his tests. If he can make topical tests, how about making a submucosal test, using any one of the allergens in solution? That may give a clue to some etiologic factor.

Dr. Albert Vander Veer, New York City.—I should like to ask whether Dr. Cohen used house dust in his tests. We find that many patients react only to that and nothing else.

Dr. Francis M. Rackemann, Boston, Mass.—I have two questions to ask. The first one is whether Dr. Cohen has tried applying dry pollen to the middle turbinate; and the second, whether in treating patients with negative skin tests, he still finds the doses given under the skin will produce local reactions.

DR. GEORGE L. WALDBOTT, Detroit.—I should like to ask two questions. Has Dr. Cohen used this method in determining sensitivity to nonprotein containing substances such as novocaine, aspirin, carbohydrates, and similar materials which do not usually give positive skin reactions? I have recently used this method with some modification in technic for the purpose of determining sensitization to local anesthetics and obtained information which I was not able to obtain with skin tests and passive transfer.

Can these reactions always easily be controlled by applying epinephrine to the nasal mucosa? I should think that there is a chance of producing very severe vasomotor rhinitis lasting for a longer period of time than desired, by a single contact with an allergen for as long as twenty minutes.

DR. RUDOLPH.—The use of this test has resulted in marked improvement in a reasonable percentage of patients in whom we had failed to find relief by other methods.

In our experience diet does not affect the number of patients referred to by many writers on this subject. We find that after the first five years of life the inhalant factors are on the ascent until about forty years when perhaps factors of infection become more important.

There were many substances to which patients in this series reacted. House dust was an important one; feathers, cotton, orris root, and wool. There were several other substances from the patients' environment. Pollen was used in this series but not because of any seasonal incidence, since all of these patients were perennial ones.

Substances other than those mentioned above are being tried. Such substances as volatile oils, foods, molds, and bacteria, but details in dilutions and technic are just being worked out.

In conclusion, we should like others of you who care to, to try this method in order that we may all benefit by the information so learned.

PERENNIAL HAY FEVER FROM INDIAN GUM (KARAYA GUM)*

STEARNS S. BULLEN, M.D.
ROCHESTER, N. Y.

THIS case is reported because, so far as I can learn, no reports of hypersensitiveness to Indian gum have appeared in the literature.

A woman, aged twenty-six years, stated that she had had a "cold" almost constantly for the past one and one-half years. Her symptoms were conjunctival redness and itching, with lachrymation; marked nasal obstruction, at times affecting both sides of her nose, and at others only one side or the other. There was a profuse watery nasal discharge, which never became thick and purulent. Prolonged paroxysms of sneezing occurred several times daily. In addition, she had a marked sensation of itching in the roof of the mouth and throat, at times extending to the ears. She never had fever or generalized aching with her "colds," and otherwise her health was good.

The rhinologist from whom she sought relief found that the nasal obstruction was caused by marked swelling of the mucous membrane covering the turbinates. This membrane showed definite pallor and appeared edematous. When the mucous membrane was shrunken, there was ample airway through her nose. The accessory sinuses showed no evidence of disease. From the history and the characteristic appearance of the nasal mucosa, a diagnosis of allergic coryza was made.

With this diagnosis in mind, questioning elicited the story that she was likely to be much worse each time she had her hair waved, which was done every two weeks. She recalled that on some occasions, when the wave-set material had happened to get on the skin of her neck or face, small urticarial wheals had appeared and itched intensely for a few minutes. No such wheals ever appeared on her scalp.

Some wave-set materials failed to make her symptoms worse. She used one such material at home to make minor adjustments to her hair daily.

It was learned that the basis for most wave sets are emulsions of Indian gum, gum tragacanth, gum acacia, and the gum of flaxseed and quince seed.

Skin tests, by the scratch method, were done on her forearm with dry powders of these substances, as well as with the emulsion which had

*Read before the Tenth Annual Meeting of the Society for the Study of Asthma and Allied Conditions, Atlantic City, N. J., April 28, 1934.

aggravated her symptoms, and the one which had produced no symptoms. In addition, she was tested with orris powder, the animal epidermals, etc.

Within three minutes after the application of the Indian gum to the scratch, there occurred marked itching at the site of the test; a wheal 2 cm. in diameter appeared shortly, with pseudopods. In fifteen minutes red streaks developed extending from 10 cm. to 12 cm. up the arm. There was a similar reaction with the preparation which had produced symptoms, but it was somewhat smaller and appeared more slowly. No reactions occurred with any of the other substances tested.

It was learned later that the material which had produced hives on her face and neck and made her nasal and eye symptoms worse, was a decoction of Indian gum; whereas, the one which she had been able to use without symptoms was prepared from quince seed.

Following her abstinence from the use of the Indian gum preparation on her hair, her symptoms gradually disappeared so that at the end of ten days her "cold" was entirely well. She has remained symptom free for over three months with the exception of one episode of her characteristic coryza after a so-called "tonic wave" had been put in her hair. She was assured that the material used for this contained no Indian gum, but it was found later that it did. Her symptoms ceased within twenty-four hours after she washed this material out of her hair.

Cooke's postulates seem to have been fulfilled in this case in that: (1) A reaction was produced on the skin with the suspected material. (2) It could be shown that the patient had been exposed to this substance. (3) Her symptoms had been reproduced when she was exposed to the substance.

Twenty other persons have been tested with Indian gum in the same way with entirely negative results. This seems to rule out the possibility of some irritating material in the gum producing a nonspecific reaction on this patient's skin.

Indian gum, as it is sold commercially, is a fine white powder. Some of the inferior products are grayish in appearance. The United States Dispensatory[1] states that it is made from the gummy exudate of a tree (*Anogeisus Latifolia*, Wall), which is found chiefly in India. It is also known as Karaya gum and gum ghatti. It is entirely soluble in water, forming a viscous, adhesive mucilage, more viscous than acacia, for which it has the same uses in pharmacy.

For commercial uses other than those related to pharmacy, the gummy exudate from other trees seems to be used, and may be called by the same names. The Scientific American Supplement[2] states that gum from the trees *Sterculia Urens*, Roxb., *Sterculia Villosa* and *Cocklospermum gossypum* has the same qualities.

In the same article, it is stated that the following names are sometimes given to this product: Sterculia gum, Kadaya gum, Karai, Katira, Katila, Karaya, Kuteera, Kutira and Kutera, Kawali, pandruk, loli, gula, Kolu, penari and Velley-pentali.

Wave-set materials must be mucilaginous in character to hold the hair in place after it is "waved." A solution of Indian gum serves this purpose admirably. In addition, alkaline carbonates or borax are usually added because they react with the sebum to form a species of soap which helps to hold the hair in place. Some of the products are perfumed.

The sticky material is either applied to the hair with a brush, or is poured on the air and worked in with the hands or a comb. After the hair is suitably waved it is held in place with a net or combs, while it is dried with hot air. When the hair is dry, a variable amount of flaky powder remains attached to it. Some of this material is sure to fall off each time the hair is combed.

Indian gum is also used in some foods, such as pies of the soft custard variety, to make them hold their shape when cut, and in some ice creams. If ice cream tends to holds its form as it melts, it probably contains Indian gum.

Asthma resulting from sensitization to flaxseed used in a wave-set material has been reported by Grant.[3] I have seen in the past three years twelve flaxseed-sensitive patients whose symptoms (asthma and allergic coryza) were caused by similar preparations.

Materials used for wave sets deserve consideration, as well as orris powder and other substances used in cosmetics, as potential causes of allergic manifestations.

I wish to thank Mr. Frank H. Goler for much kindly assistance.

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DISCUSSION

DR. HORACE S. BALDWIN, New York City.—Dr. Bullen's paper is chiefly of interest because it describes another substance to which patients may become sensitive.

The wide use of hair lotions containing flaxseed, acacia, quince seed, Indian gum, and other possible atopens, will undoubtedly increase the number of patients troubled by their use. So far as I know, no successful substitute for these substances in wave set has as yet been found. It would be interesting to know whether Dr. Bullen or any other member of the Society is familiar with any nonallergic wave set preparation.

Dr. Bullen stresses the important rôle of flaxseed sensitivity. During the past year I have had an asthmatic patient who is skin sensi-

tive to quince seed. She used a hair lotion containing quince seed, but there was no considerable amount of trouble due to its use of which the patient was aware.

Acacia has long been used as a neutral and supposedly inert vehicle. Last year before this Society Dr. Spielman and I reported a definite case of atopic sensitivity to acacia, and if it continues to be used in wave sets, I have no doubt other cases of sensitivity to this will be found. The fact that these gums are listed as polysaccharides is on the surface misleading because in the case of acacia 0.5 per cent of total nitrogen is present and it also gives a positive biuret test.

SKIN REACTIONS IN INFANTS*

Susceptibility of the Skin of the Newborn to Passive Atopic Sensitization: Comparison With Reactions to Histamine

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Baltimore, Md.

THIS study was undertaken in order to determine the age at which children might be expected to react positively to intradermal testing with atopen. As infants during the first weeks or months of life rarely exhibit clinical evidence of allergy of a type commonly associated with an immediate skin reaction, passive transfer technic was used, in order that a sufficient number might be tested.

Method.—Serum was obtained from patients sensitive to a 1:100,000 dilution of short ragweed pollen extract, containing approximately 60 mg. of nitrogen per 100 c.c., and prepared by extracting 5 gm. of dry pollen in 100 c.c. of Coca's solution. Two, three, or four sites in the infants' left forearms and arms were sensitized by intradermal injection of 0.05 c.c. of serum.

The toughness and very fine texture of the infants' skins led to some difficulty in locating all injections in the same dermal layer; they were given as superficially as possible. Occasional leakage due to struggling of the subjects led to slight inaccuracy in dosage. All injections were made with No. 27, ½ inch needles, and tuberculin syringes.

Twenty-four hours after sensitization, these sites, together with control sites in the right arms and forearms, were tested with varying dilutions of short ragweed extract prepared as above. Dilutions varied from 1:100 to 1:10,000 of standard extract; 0.02 c.c. was used in each test. Reactions were read in fifteen minutes.

A preliminary series of children, ranging in age from eight days to eight months, was tested to determine the age at which passive sensitization could be accepted. As infants in the first two weeks of life were found to react definitely, the work was confined to infants of this age.

The type of reactions noted did not differ essentially from that seen in older children and adults. With higher concentrations of atopen, wheal formation occurred, together with a surrounding zone of erythema. Redness of the skin, and jaundice in a few subjects, made the reactions rather difficult to read, though usually the erythema was intense

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enough to stand out against the surrounding skin; wheals could be accentuated by gentle tension. Greater dilutions of the test atopen frequently caused erythema without whealing. Pseudopods were not observed in any reactions. Wheals were usually quite small, varying from 0.3 to 1.2 cm. in diameter. Erythema less than 1.5 cm. in diameter without whealing, was considered a negative reaction.

TABLE I

SUBJECT NO.	AGE DAYS	DILUTION OF SHORT RAGWEED EXTRACT USED							
		1:100		1:1,000		1:5,000		1:10,000	
		R	L	R	L	R	L	R	L
1	3			W-0 R-0	W-0.5 R-3.0	W-0 R-0	W-0 R-0		
2	8					W-0 R-0	W-0 R-0	W-0 R-1.0	W-0 R-0
3	12			W-0 R-0	W-0 R-2.0	W-0 R-0	W-0 R-3.0	W-0 R-0	W-0 R-1.5
4	3			W-0 R-1.2	W-0.3 R-2.0	W-0 R-1.0	W-0 R-1.0		
5	6			W-0 R-0	W-0.5 R-2.0	W-0 R-0	W-0 R-0		
6	4					W-0 R-3.0	W-0.5 R-3.0	W-0 R-±	W-0.3 R-1.5
7	3					W-0 R-0	W-0.6 R-2.3	W-0 R-0	W-0.3 R-1.8
8	8			W-0 R-0	W-1.0 R-2.0	W-0 R-0	W-0 R-1.5	W-0 R-0	W-0 R-1.5
9	4			W-0 R-0	W-0.9 R-2.3	W-0 R-0	W-0.4 R-2.0	W-0 R-0	W-0 R-0
10	6	W-0 R-0	W-0.9 R-2.5	W-0 R-0	W-0.3 R-2.5	W-0 R-0	W-0 R-1.5	W-0 R-0	W-0 R-0
11	11	W-0 R-0	W-0.9 R-2.5	W-0 R-0	W-0.3 R-2.5	W-0 R-0	W-0 R-0		
12	3	W-0 R-0	W-0.8 R-2.0	W-0 R-0	W-0.5 R-2.5	W-0 R-0	W-0 R-0	W-0 R-0	W-0 R-0
13	3	W-0 R-0	W-0.5 R-2.0	W-0 R-0	W-0.8 R-2.5	W-0 R-0	W-0 R-0	W-0 R-0	W-0 R-0
14	4	W-0 R-0	W-0.8 R-3.0	W-0 R-0	W-0.4 R-1.5	W-0 R-0	W-0 R-1.5	W-0 R-0	W-0 R-0
15	2	W-0 R-0	W-0.9 R-2.0	W-0 R-0	W-0.4 R-2.0	W-0 R-0	W-0 R-2.0	W-0 R-0	W-0 R-1.8
16	2	W-0 R-±	W-1.0 R-2.5	W-0 R-±	W-0.7 R-3.0	W-0 R-±	W-0.4 R-3.0	W-0 R-±	W-0.3 R-1.8
17	5	W-0.4 R-0	W-1.2 R-2.5	W-0 R-0	W-0 R-1.5	W-0 R-0	W-0.5 R-2.0	W-0 R-0	W-0 R-±
18	14 hr.	W-0 R-±	W-1.2 R-2.0	W-0 R-0	W-0.4 R-2.0	W-0 R-0	W-0 R-2.0	W-0 R-0	W-0 R-0

Figures indicate diameter of reactions in centimeters.
W = Wheal
R = Redness or Erythema
Tests – left arm (L)
Controls – right arm (R)

Eighteen infants were tested. Fifteen of these reacted positively, but there was great variation in the size of the wheal and erythema in different infants tested with the same serum and dosage of atopen. (Table I.) One infant, who was tested early in the series, and reacted strongly to a 1:5,000 dilution of extract, was later negative to this

concentration when sensitization was attempted with a different serum. Two subjects reacted positively to both test and control; in each of these, the sensitized site reacted with a wheal, and the control site with erythema only (Subjects 4 and 5). Testing in these two subjects was only carried out to the 1:1,000 dilution. One infant (No. 3) reacted with erythema alone to the most concentrated test dose used; in this case 1:1,000.

It may be noted from the table, however, that in the latter part of the series, of the nine infants who were tested with 1:100 dilution of pollen extract, all responded with strong wheal and erythema reactions, the corresponding controls being negative in every case, except for a small wheal in No. 17.

Because of the great variability of the reactions, and as most of them were considerably less extensive than reactions obtained with the same serums when used to sensitize adults, it was considered that the differences might be due to finer structure of the infant skin, rather than to any essential physiologic difference. To confirm this, five adults, ranging in age from twenty-five to sixty years, and six infants, ranging in age from sixteen hours to eight days, were tested with 0.02 c.c. of a 1:10,000 dilution of ergamine acid phosphate (0.002 mg). Tests were done on the flexor surface of the left forearm. Reactions in the infants were consistently much smaller than those observed in adults, wheal and erythema being about half the diameter of the adult reactions. (Tables II and III.) There was, also, much variation in the size of the reactions obtained in the infants, while the adult reactions were fairly uniform.

TABLE II

REACTION OF ADULT SKIN TO HISTAMINE (ERGAMINE ACID PHOSPHATE 0.002 MG.) INTRADERMALLY

SUBJECT NO.	DIAMETER OF REACTION IN 10 MINUTES (CM.)	
	WHEAL	ERYTHEMA
1	1.0	7.0
2	1.3	5.0
3	1.3	5.5
4	1.0	6.0
5	1.2	4.5
Average	1.13	5.6

This difference in reaction to histamine probably explains the small size of the wheals and erythema seen in sensitized infants when tested with ragweed extract. McConnell[1] has shown that variations in response to histamine and to atopens are almost exactly parallel when tests are carried out on asthmatic individuals during and between attacks.

Skin reactions have not been studied very extensively in small infants. Bell and Eriksson[2] reported testing the skins of newborn infants whose mothers demonstrated clinical symptoms of atopy, with positive skin tests and atopic reagins in the blood. All of these newborn infants gave negative skin reactions; neither could atopic reagins be demonstrated in the cord blood at birth, nor in venous blood in the first ten days after birth.

TABLE III

REACTION OF INFANT SKIN TO HISTAMINE (ERGAMINE ACID PHOSPHATE 0.002 MG.) INTRADERMALLY

SUBJECT NO.	AGE	DIAMETER OF REACTION IN 10 MINUTES (CM.)	
		WHEAL	ERYTHEMA
1	8 days	0.8	3.0
2	9 days	0.7	3.0
3	30 hours	0.4	1.5 (?)
4	16 hours	0.4	2.5
5	4 days	0.6	2.0
6	4 days	0.4	1.5
	Average	0.55	2.2

Von Gröer[3] in 1924, tested many subjects of all ages with intracutaneous injections of morphine hydrochloride, and calculated a "lymphagogic index" by multiplying the diameter of the resultant wheal (in millimeters) by the reciprocal of the logarithm denoting the concentration of the solution. Thus a 20 mm. wheal produced by a 1:10,000 dilution would indicate a "lymphagogic index" of $4 \times 20 = 80$. He found that the lymphagogic index determined by this method was about 50 in the newborn, rising in the second half-year to 60-80, where it remained until puberty, increasing then to 70-100.

Adelsberger[4] in 1927, tested the skins of infants of different ages to various irritants, applied by patch and intradermal tests. The youngest infants were much less susceptible to patch tests with substances likely to produce irritation than were the older children. The youngest infants were also much less sensitive to intradermal injections of foreign protein (horse serum). Older sucklings and children in the first few years of life showed a growing susceptibility. This author discussed the possible causes of the nonreactivity of the skin of the newborn, finally concluding that it is due to an anatomic difference.

CONCLUSIONS

1. Newborn infants can be passively sensitized to ragweed by the Prausnitz-Küstner method.

2. When higher concentrations of test solutions are used, the reactions resemble those observed in adults.

3. Marked variations are noted in the dose of atopen required to produce reactions of the same intensity in different infant skins when the same serum is used for sensitization.

4. The smaller and variable reactions noted in the skin of the newborn, when tested in this manner, correspond closely to the small and variable reactions following intradermal injection of histamine.

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THE RELATION OF INSPIRATORY DISTENTION OF THE LUNGS TO EMPHYSEMA*

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A REVIEW of the literature reveals much confusion concerning the relationship of bronchial obstruction to emphysema. It is my purpose to endeavor to answer two questions which have been the source of much argument: (1) Does bronchial obstruction cause emphysema? (2) If it does, by what mechanism is the emphysema produced?

LITERATURE

Bronchial obstruction as a cause of emphysema has been considered from both a clinical and an experimental point of view.

It is well known that asthmatics have emphysema. Alexander, Luten and Kountz[1] found emphysema in 49 out of 50 patients suffering from asthma, and Huber and Koessler[2] found evidence of emphysema in each of the six asthmatic patients whom they studied carefully at necropsy. Hoover and Taylor,[3] in 1915, made the observation that alveolar air, taken from dogs by manual compression of the chest and abdomen, could be increased greatly in amount if breathing were obstructed for some time before the air was removed. They concluded that stenosis anywhere in the tracheobronchial system will cause an increase in residual air in that part of the lungs supplied by the affected air passages. However, in 1922, Hoover[4] concluded from further experimentation that bronchoconstriction does not cause emphysema. In this experiment Hoover placed the entire animal in an air-tight box with a tracheal cannula leading outside. The box was connected to a Benedict apparatus, and the size of the respiratory excursions was taken as an indication of the changes in the pulmonary volume. He injected histamine for bronchoconstriction but failed to produce marked pulmonary distention. However, he found emphysema in cases where one bronchus was obstructed, and concluded that although the cause of emphysema was not clear, it might be caused by the unequal distribution of expiratory stenosis but not by generalized bronchoconstriction.

Loeb[5] placed a plug which produced tracheal stenosis in dogs. He stated that emphysema did not result. Harris and Chillingsworth,[6]

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Read before the American College of Physicians, San Francisco, February, 1932.

however, claim to have produced emphysema by an almost identical method, as have Kountz and Alexander.

Cloetta[7] placed an isolated cat's lung in a glass jar to which a vacuum pump was connected. He observed that by eliminating the negative pressure in the jar, the lung returned to its resting size. However, if the animal had received an injection of histamine previously so that the bronchi were constricted the pulmonary volume was greatly increased with normal pressure changes.

Regarding the second question concerning the mechanism by which bronchial obstruction produces emphysema, there are two main theo-

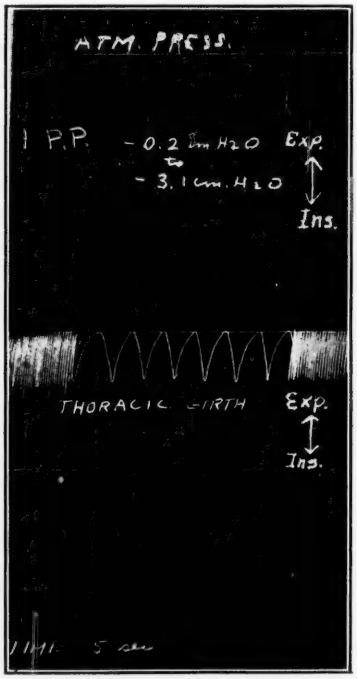


Fig. 1.—Kymographic tracing of intrapleural pressure and respiratory movement (pneumographic tracing). The downstroke in both tracings is made on inspiration and the upstroke on expiration. The intrapleural pressure (I.P.P.) is subatmospheric.

ries: (1) the inspiratory theory originally advocated by Laennec[8] and supported by Hofbrauer[9]; (2) the expiratory theory which was originated by Biermer[10] and which has received the most support. Biermer believed that with bronchoconstriction there is active expiratory effort which compresses the lung and raises intraalveolar pressure which in turn weakens the alveolar wall and produces emphysema. There has been little actual experimental work on this problem. Auer and Lewis[11] first found more negative intrapleural pressure in guinea pigs in anaphylactic shock. Smith and his coworkers[12] also found a more

negative intrapleural pressure in guinea pigs until pulmonary distention was extreme and the obstruction was complete, when the pressure became positive.

EXPERIMENTAL

We investigated these problems by studying the effects of bronchoconstricting drugs on the thoracic girth which indicated the degree of pulmonary distention and on the intrapleural pressure of dogs. The

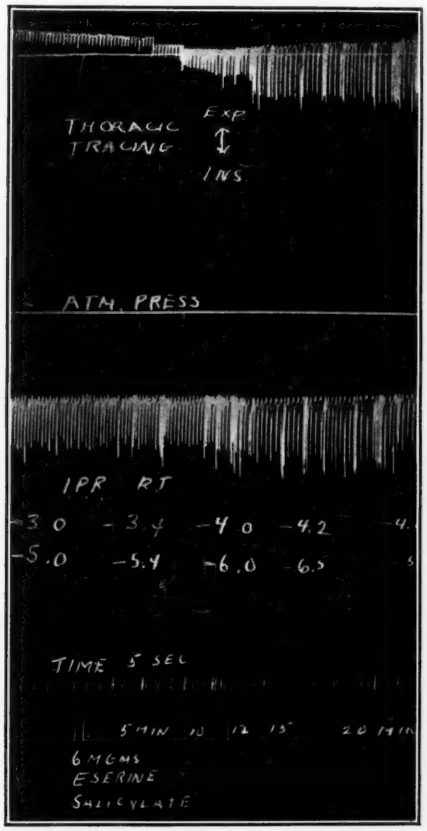


Fig. 2.—Pneumograph showing the effects of an injection of eserine on the intrapleural pressure and thoracic girth of a dog lightly anesthetized with sodium amytal. Intrapleural pressure figures are indicated in centimeters of water.

animals were anesthetized lightly by means of sodium amytal intraperitoneally. A trocar was placed in the pleural cavity, connected to a "Y" tube of which one branch led to a manometer and the other to a kymograph. The thoracic girth was measured by means of a steel tape and records were obtained by an Ellis pneumograph which indicated the relative inspiratory or expiratory position of the chest. Fig. 1 shows what may be considered a normal curve.

We found that after the administration of such bronchoconstricting drugs as eserine or pilocarpine, there was a mean increase in thoracic girth and a more negative intrapleural pressure.[13] (Fig. 2.) When we used histamine, the animals were thrown into profound shock, and the respiratory movements were so disturbed that no conclusions could be drawn.

In order further to determine the effect of bronchoconstriction on the relative inspiratory and expiratory strength of respiration in clinical conditions, intrapleural pressure determinations have been made in four asthmatic individuals. In all cases during an asthmatic attack it was found that the intrapleural pressure was more negative than normal instead of more positive as is usually believed.

CASE 1.—C. B. During a severe asthmatic attack the patient's intrapleural pressure was –12 cm. to –16 cm. of water. After the asthmatic attack was over and the patient was breathing quietly, the pressure was –2 cm. to –5 cm.

CASE 2.—Mrs. K. The initial intrapleural pressure (without asthma) was –3 cm. to –6 cm. of water; after two injections of 550 c.c. of air, the pressure was 0 cm. to –2.5 cm. The patient then developed bronchial asthma and in spite of the therapeutic pneumothorax, the intrapleural pressure fell to –3 cm. to –8 cm. The average intrapleural pressure following the onset of asthma was 4 cm. more subatmospheric than before the onset of asthma.

CASE 3.—Mrs. O. suffered from bronchial asthma, emphysema, and pulmonary tuberculosis. She was found to have an intrapleural pressure of –5 cm. to –10 cm. of water during an asthmatic attack. Aron[14] most accurately determined normal intrapleural pressure in a healthy individual. His average of 36 determinations was –4.64 mm. Hg at inspiration and –3.02 mm. Hg at expiration. This corresponds respectively to –6.3 cm. to –4.1 cm. of water. Thus, this patient had an average pleural pressure of 2.5 cm. of water more subatmospheric than normal during the asthmatic attack, in spite of having emphysema, which is known to cause a more positive intrapleural pressure because of a loss of pulmonary elasticity.

CASE 4.—L. T. The intrapleural pressure during a mild asthmatic attack was –2 cm. to –11 cm. of water. This is an average of 1.3 cm. more negative than Aron's average in a normal person, in spite of the fact that this patient had severe emphysema.

Kountz[15] has found similar change in intrapleural pressure during the asthmatic attack.

In these first experiments, we have endeavored to show that bronchoconstriction may cause pulmonary distention, and during the process of development of pulmonary distention there is a more negative intra-

pleural pressure. We have been interested in correlating the observations we have made. Why does bronchoconstriction cause pulmonary distention and a more negative intrapleural pressure?

TABLE I

EFFECTS OF THE INHALATION OF 10 PER CENT CARBON DIOXIDE IN OXYGEN ON INTRAPLEURAL PRESSURE AND THORACIC GIRTH IN A DOG WEIGHING 17 KILOS LIGHTLY ANESTHETIZED WITH SODIUM AMYTAL

	INTRAPLEURAL PRESSURE IN CM. H_2O		THORACIC GIRTH	
	INSPIRATION	EXPIRATION	INSPIRATION	EXPIRATION
			cm.	cm.
Normal	−4.5	+0.5	54.6	54.2
CO_2 inhaled	−8.0	+0.5	55.3	54.2
CO_2 off	−6.5	+0.5	55.0	54.2

It has been shown that with bronchoconstriction and with increased resistance to respiration there is an accumulation of carbon dioxide and anoxemia.[16] We therefore studied the effects of the direct administration of carbon dioxide[17] on the intrapleural pressure and thoracic girth

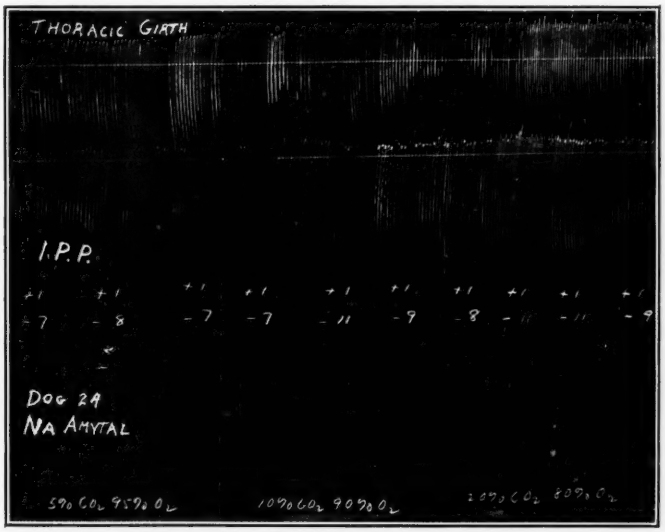


Fig. 3.—Effects of different concentrations of carbon dioxide on intrapleural pressure and thoracic girth in a dog under light sodium amytal anesthesia.

of dogs. We obtained consistent findings, namely, a more negative intrapleural pressure and an increase in the mean thoracic girth. (Fig. 3 and Table I.) With high concentrations of carbon dioxide the intrapleural pressure finally becomes more positive than normal, but the increase is not so marked as the decrease. Anoxemia was produced by rebreathing through a spirometer, the carbon dioxide being absorbed by placing lime in the circuit. Alveolar air and blood gas analyses showed that there was no accumulation of carbon dioxide and that the

percentage of oxygen had decreased gradually to as little as 8 per cent.[18] Anoxemia has much the same effect as increased carbon dioxide tension. (Fig. 4 and Table II.) There gradually develops a more negative intrapleural pressure and an increase in mean thoracic girth until the anoxemia becomes extreme, after which active expiratory effort ensues and the intrapleural pressure becomes slightly positive just as it does with high concentrations of carbon dioxide.

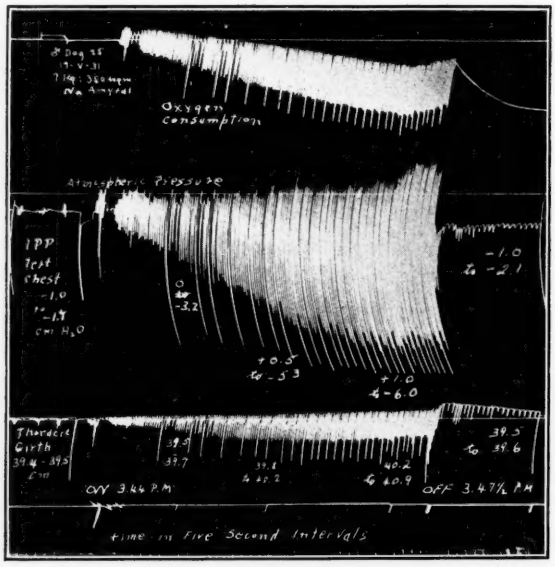


Fig. 4.—Effects of anoxemia on intrapleural pressure and thoracic girth in a dog under sodium amytal anesthesia. The upper tracing indicates the gradual consumption of oxygen from a Grogh's spirometer. Carbon dioxide was absorbed by placing soda lime in the circuit. Intrapleural pressure figures are in centimeters of water. Thoracic girth was measured by a steel tape.

TABLE II

EFFECT OF ANOXEMIA ON INTRAPLEURAL PRESSURE AND RELATED FUNCTIONS IN A DOG WEIGHING 12 KILOS ANESTHETIZED WITH 625 MG. OF SODIUM AMYTAL

	INTRAPLEURAL PRESSURE IN CM. WATER	THORACIC GIRTH IN CM.	RESPIRATORY RATE PER MINUTE	O_2 TENSION MM. MERCURY
Normal	−2.0 to −5.0	46.8 to 46.88	39	156
1 minute	−2.0 to −6.0	46.9 to 47.0	42	105
2 minutes	−1.0 to −8.0	47.1 to 47.2	46	73
2½ minutes	+2.0 to −6.0	47.0 to 47.1	23	53

In order to test the hypothesis that decreased intrapleural pressure may cause emphysema, normal rats were placed in special tanks in which the barometric pressure could be reduced. The pressure was slowly reduced from the normal (760 mm. of mercury) to 350 mm. to 450 mm. of mercury. This corresponds to about 60 mm. of oxygen tension instead of the atmospheric 150 mm. and is equivalent to about 8

per cent oxygen instead of the usual 20 per cent. At 60 mm. oxygen tension we found a more negative intrapleural pressure in dogs in acute experiments. After periods of from one to ten weeks, the rats were removed and sections of their lungs were made (Fig. 5). It may be seen that the alveoli are greatly distended and their walls are so thin and broken in places that two or more alveoli may have coalesced. Campbell also found emphysema in experimental animals,[19] using a similar method.* Hurtado[20] carefully studied the inhabitants living in the Peruvian Andes at an altitude of 14,890 feet, where the average barometric pressure was 410 mm. Hg. He found that from early child-

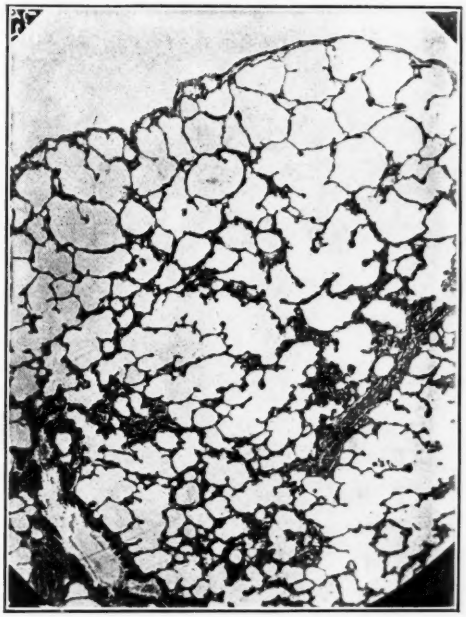


Fig. 5.—Rat lung (×50) after two months under 350 mm. of mercury pressure. This corresponds to about the degree of anoxemia found in Fig. 4.

hood to old age, the native presents a remarkable thoracic development as determined by the large thoracic girth and the low position of the diaphragm. He found a wide subcostal angle, a hyperresonant percussion note, a diminution in the area of cardiac dullness, and an increase in the pulmonary second sound. There was no diminution in vital capacity. Autopsies on animals and on 3 natives showed dilated pulmonary alveoli, but the most outstanding pathologic finding was congestion of the pulmonary capillaries. Hurtado considers the above condition as a physiologic emphysema, and thinks it differs from true emphysema. Most of our animals were placed in a lower atmospheric

*Christie recently has also shown this to be true using an identical method. J. Clin. Investigation 8: 295, 1934.

pressure (350 mm.) than that in the Peruvian Andes (410 mm.), and this probably accounts for the more marked emphysema.

DISCUSSION

Normally, intrapleural pressure is subatmospheric because of the stretch on the elastic lung tissue. During inspiration, as the lung is further stretched or distended by enlargement of the thorax, the pressure becomes more negative. During expiration, there is less distention of the lung as a result of relaxation of the muscles of inspiration so that the pressure becomes less negative. In order that intrapleural pressure may rise above atmospheric pressure in the normal animal, there must be active expiratory effort. If, by a greater inspiratory effort than normal, the lungs are more distended than usual, the pressure should become more negative than normal. A more negative intrapleural pressure in these experiments must therefore mean greater inspiratory effort.

It is important to distinguish pulmonary distention from emphysema. Pulmonary distention indicates an increase in air in the normal lung with retention of elasticity. Since the lung is abnormally stretched, the intrapleural pressure is more negative than normal. Pulmonary distention is usually a physiologic mechanism to increase ventilation and is found to occur after severe physical exercise, after ascent to high altitudes, in pneumonia, etc. The familiar "compensatory emphysema" is an example of pulmonary distention. We have shown in the above experiments that pulmonary distention occurs following bronchoconstriction, anoxemia, and increased alveolar carbon dioxide tension. In emphysema, on the other hand, there is also an increase in the air in the lung, but associated with this there is loss of elasticity. Consequently, the intrapleural pressure is less negative than normal, and there is respiratory insufficiency. Pathologically there is distention of the alveoli with thinning of their walls and frequent coalescence of alveoli. It may be reasonably assumed that pulmonary distention if long continued will lead to true emphysema. We have shown that anoxemia produces pulmonary distention in an acute experiment. Prolonged anoxemia was found to produce true emphysema. The first asthmatic attack produces pulmonary distention. It is only after repeated attacks that true emphysema gradually results.

In our review of the literature we have shown that there is abundant evidence both clinical and experimental that bronchoconstriction produces pulmonary distention and emphysema. The most outstanding negative results were obtained by Hoover.[4] We used practically the same technic that Hoover used and obtained evidence of pulmonary distention. We believe that the discrepancy between our results and those obtained by Hoover can be explained by the fact that Hoover used histamine. When we used histamine, we threw our dogs into

profound shock and the respiratory movements were so disturbed that no conclusions could be drawn. Using other bronchoconstricting drugs, however, pulmonary distention was found to occur (Fig. 2).

If, then, bronchoconstriction can produce pulmonary distention and subsequent emphysema, as our experiments indicate, the next question arises, by what mechanism is the emphysema produced? By far the most widely quoted hypothesis is that originated by Biermer in 1870. Biermer believed that with broncoconstriction there is an active vigorous expiratory effort which compresses the lung and raises intra-alveolar pressure, which in turn weakens the alveolar wall and emphysema results. Inasmuch as intrapleural pressure can be used as a measure of relative inspiratory and expiratory strength, a more negative intrapleural pressure following bronchoconstriction means greater inspiratory muscular effort. The lungs are not compressed by an active expiratory effort as Biermer believed, but are distended by an active inspiratory effort, as illustrated in Fig. 2 by the lowered intrapleural pressure. How active expiration and more positive intrapleural pressure can cause generalized pulmonary distention or emphysema, is difficult to understand. Logically it would appear to have an opposite effect, that is, it would cause a diminution in the size of the lungs. There is one exception to this. With vigorous active expiration as in coughing, the captivated air goes to those parts of the lung which have the least support, the apex and the periphery. Thus, emphysematous blebs may be formed in these areas.

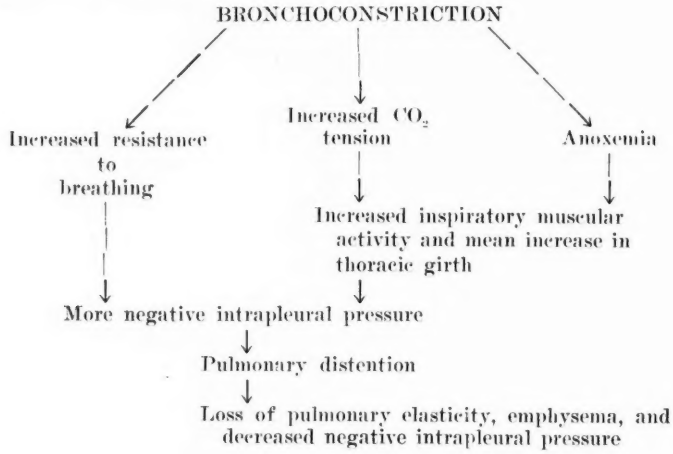
Many clinicians have stated that there is active expiratory effort during the asthmatic attack. This is not true in the ordinary asthmatic attack. In asthma, expiration is greatly prolonged but it is passive. Inspiration is short, active, and violent. In extreme dyspnea, however, active expiratory effort usually ensues. Piness[21] has clinically confirmed these findings. In severe emphysema with complete loss of pulmonary elasticity, the intrapleural pressure is less negative than normal and may rise above atmospheric pressure during expiration. Thus active expiration is usually necessarily present in these cases, especially when the intrapleural pressure is above atmospheric pressure. This should not be confused with the response to bronchoconstriction in the early stages of the production of emphysema where we have shown that the main respiratory effort is inspiratory.

With bronchoconstriction and increased resistance to respiration there is diminished expansion of the lung with ordinary or increased respiratory effort. This produces a greater stretch on the lung tissue and a more negative intrapleural pressure. Thus when the trachea of an animal is clamped, the intrapleural pressure becomes more negative on the first respiratory effort. Later, accumulation of carbon dioxide and anoxemia can be expected to occur with bronchoconstriction. We have shown by direct experiment that increased alveolar

carbon dioxide tension and anoxemia cause an increased mean thoracic girth and more negative intrapleural pressure. It therefore appears that there are three factors causing the decrease in intrapleural pressure and pulmonary distention after bronchoconstriction: (1) increased resistance to respiration and diminished expansion of the lung; (2) accumulation of carbon dioxide; (3) anoxemia. The first factor is always present in the asthmatic attack. The other two are probably present mainly in the more severe attacks.

In severe emphysema, as has been pointed out, the intrapleural pressure is more positive than normal. This is after the decreased intrapleural pressure has overstretched the elastic tissue so that the lungs lose their elasticity and the intrapleural pressure is more positive. Kountz, et al.,[22] have shown this sequence of events by means of a ball-valve tracheal cannula in dogs. They measured the intrapleural pressure as the emphysema was gradually produced and found a rise in intrapleural pressure as the elasticity of the lung was decreased. Smith and his coworkers showed the same sequence of events during anaphylactic shock in the guinea pig. They found a more negative intrapleural pressure as the process of distention was going on. When the pulmonary distention was complete and no more air could be forced into the lungs, the pressure then became positive when active expiratory effort ensued. The mechanism by which emphysema is produced may then be considered an example of a physiologic process which has been present for such a long period of time as to cause pathologic changes. The clinical picture of emphysema with loss of pulmonary elasticity may be considered the end-result of this process.

The pathogenesis of emphysema following bronchoconstriction may be represented as follows:


BRONCHOCONSTRICTION
Increased resistance to breathing
Increased CO_2 tension
Anoxemia
Increased inspiratory muscular activity and mean increase in thoracic girth
More negative intrapleural pressure
Pulmonary distention
Loss of pulmonary elasticity, emphysema, and decreased negative intrapleural pressure

The result of these experiments may help to clarify some of the various etiologic factors which are known to produce emphysema. It

is stated that people who have done hard physical labor or indulged in vigorous athletic sports for long periods of time are more prone to develop emphysema than sedentary workers. During severe muscular exertion anoxemia and retention of carbon dioxide may be expected to occur. If this continues over long periods of time, emphysema should result just as it does with bronchoconstriction. Dwellers at high altitudes have been known to develop emphysema. In these cases anoxemia with resulting decreased intrapleural pressure is probably responsible. It has been suggested that another factor in the production of emphysema following anoxemia may be the malnutrition of the elastic tissue with subsequent loss of elasticity. In chronic bronchitis there is obstruction to respiration by the inflammatory exudate and by edema, and the mechanism of the production of emphysema is similar to that following bronchoconstriction.

Although there is a similarity in the pathogenesis of the emphysema due to bronchial obstruction and that due to the other causes mentioned above, there are definite differences which need to be pointed out. In bronchial obstruction after the pulmonary distention is complete, little air can get in and out of the lungs. Thus, they remain more distended for longer periods of time than in the nonobstructive types. Furthermore, with bronchial obstruction there is a greater stretch on the lung tissue from increased resistance to respiration with lowering of intrapleural pressure. This condition does not exist in the nonobstructive types. These factors explain why there is a greater severity and incidence of emphysema from bronchial obstruction than from the other causes mentioned above.

SUMMARY

1. Experimental bronchoconstriction in dogs first causes pulmonary distention with increased inspiratory position of the chest, and a more negative intrapleural pressure.

2. In four cases of bronchial asthma, the intrapleural pressure was found to be more negative than normal instead of more positive, indicating active pulmonary distention by increased inspiratory effort.

3. The prolonged effect of more negative intrapleural pressure causes distention and stretching of the lungs with probable loss of elasticity. This is the first step in the production of structural emphysema. Thereafter, the intrapleural pressure rises, and the weakened alveolar walls subjected to further strain rupture and coalesce.

4. Direct experiments show that increased carbon dioxide tension and anoxemia also cause a more negative intrapleural pressure and a mean increased thoracic girth.

5. Anoxemia produced by very low oxygen tensions has been shown to produce emphysema in rats.

6. The greater negative intrapleural pressure found in bronchoconstriction may be explained by increased resistance to respiration, carbon dioxide retention, and anoxemia resulting from bronchoconstriction.

I desire to thank Dr. C. D. Leake for his aid and advice; Dr. F. S. Sundstroem and Mr. George Girogossiantz, who loaned their low atmospheric pressure tanks; and Dr. Sydney I. Shipman, who measured the intrapleural pressure in Cases 2 and 4.

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A STUDY OF THE SILK ALLERGEN*

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IN 1923, Clarke and Meyer[1] reported a case of asthma which they attributed to the gum or glue adherent to the silk fiber. Figley and Parkhurst[2] too believe that the gum or glue in the raw silk acts as the atopen and that symptoms are caused principally by inhalation. They also reported constitutional reactions resulting from inoculations of their silk extract.

For many years, we have had the clinical experience of seeing many positive skin reactions to silk extracts, in using the scratch or intracutaneous method. Diagnosis based upon such dermal reactions gave rise to so many disappointments in clinical results that it was thought advisable to discard it. However, the recent report of Figley and Parkhurst[2] has led us to believe that perhaps we may be dealing with a true and potent atopen, but for some unknown reason we have not encountered it in our practice.

It is our intention, therefore, to report our observation of the relative frequency of positive silk reactions in allergic and nonallergic persons. Skin tests with extracts obtained from several laboratories are compared. The search for the allergenic factor led to the study of the various parts of the silk cocoon, the source of the silk fiber itself and of all extracts used for skin testing. We hoped in this way to find a plausible explanation for so many nonspecific skin reactions and also throw some light on that part of the cocoon which contained the powerful reacting substance if there be any.

Any consideration of the relative frequency and value of positive skin reactions to silk extracts requires, first, a word about the extracts themselves. The testing material which we have used for about ten years has been prepared at the New York Hospital under supervision of Dr. Arthur F. Coca[3] who gives Milford credit for the observation that "an extract of the silkworm (pupa) contains at least ten times as much atopen as the cocoon" (the silk fibers) and concludes that the active principle in silk is the "worm" itself. No proof was ever offered to support this contention. This testing solution was always made by defatting the silk pupae with ether and then extracting them with buffered saline solution. In this paper, it shall be referred to as the New York Hospital silk extract. In our own laboratory, we prepared an extract,

*Tenth Annual Meeting of the Society for the Study of Asthma and Allied Conditions, Atlantic City, N. J., April 28, 1934.

using a mixture of the finely shredded silk cocoon fibers and pupa. The total nitrogen content was 1.28 mg. per c.c. Routine testing was done with a 0.01 dilution, reserving the 0.001 dilution for the cases suspected of silk sensitivity.

For the purposes of this paper, we reviewed the clinical records of 166 allergic cases under current treatment in our office and in the Allergy Department of the Buffalo Eye, Ear, Nose and Throat Infirmary —Wettlaufer Clinic. At the same time, as controls, 102 nonallergic persons were tested also intradermally with the same routine silk dilutions. It was found that 56, or 33 per cent, of the 166 allergic patients gave positive skin reactions, while 28, or 27 per cent, of the 102 nonallergic people showed similar cutaneous response. All doubtful reactions were recorded as negative.

Lest these high figures be attributed to faulty preparation of our extracts or to our method of skin testing, we proceeded to compare the skin reactions with testing solutions obtained from several laboratories. This included the New York Hospital extract. Another was the Lederle allergen[4] which is prepared like that of the New York Hospital already described. Another was secured from the Arlington Chemical Company which wrote that its silk extract is made from the raw silk which has never been processed or dyed.[5] The latter firm also markets a silk powder testing material which is prepared from the whole silk cocoon. Scratch tests with this form of allergen were made simultaneously with the others. We selected four patients who gave definite 4-plus reactions to our extract and whose history and symptoms suggested sensitivity to silk. Two of these patients, Mr. C. C. and Miss M. S., were also sensitive to the emanations of moths and butterflies. This may or may not have any significance. The results of these comparative tests are recorded in Table I. They indicate that there is little or no difference in the dermal reactions to extracts from these four sources no matter which method of testing is used. Incidentally, "silkworm" oil, made available through the courtesy of the Lederle Laboratories, gave negative scratch and patch reactions when tested on these patients.

Our studies were then directed to the silk cocoon which represents the pupal stage in the development of the mature moth. The life cycle of moths and butterflies has been under our frequent observation since 1930 when we reported several cases of hypersensitiveness to the emanations which are shed by the wings of these flies.[6] In our search for the allergenic factor in the silk cocoon, it was necessary to study separately, first, the outer casing which is made up of the silk fiber proper and its intrinsic gum or glue called "sericin." The second is the exuvia which is found inside the cocoon. It represents the outer skin of the silk pupa which it sheds probably just before emerging from the cocoon.

TABLE I

COMPARATIVE TESTS ON "SILK SENSITIVE" PATIENTS WITH SILK EXTRACTS FROM DIFFERENT SOURCES

SOURCE OF EXTRACT	NEW YORK HOSP.		LEDERLE		ARLINGTON SOLUTION		ARLINGTON POWDER	AUTHORS'	
PATIENT	INTRADERM.	SCRATCH	INTRADERM.	SCRATCH	INTRADERM.	SCRATCH	SCRATCH	INTRADERM.	SCRATCH
Mr. C. C.	++++	+	++++	+	++++	+	++++	++++	+
Miss C. D'A.	++++	+	+	– 0.1 ++++	++++	+	+	++++	+
Miss M. S.	++++	+	++++	+	– 0.1 +++	– 0.1 –	+	++++	+
Mrs. C. N.	++++	– 0.1 +++	++++	+	++++	– 0.1 +	++++	++++	– 0.1 +

Testing solutions contained 0.01 mg. N per c.c., except those retested with 0.1 solution as indicated above. Controls were made with N/10 and buffered saline solutions, respectively.

Microscopic examination of this exuvia revealed a large number of blood cells (leucocytes) and a homogeneous fluid, the insect's blood serum. (Fig. 1.) The third part of the cocoon is the chrysalis or pupa and not silkworm as some have erroneously called it. Of these three structures, none of the writers on silk sensitivity has made any mention of the exuvia as a possible factor in silk sensitivity.

Specimens of silk cocoons (*Bombyx mori*) which are prepared for the trade were graciously contributed by the Central Raw Silk Association of Japan. It should be noted that all these cocoons were heated so as to kill the chrysalis and prevent its hatching into a moth. Another batch of raw, unheated and untreated cocoons was picked from several

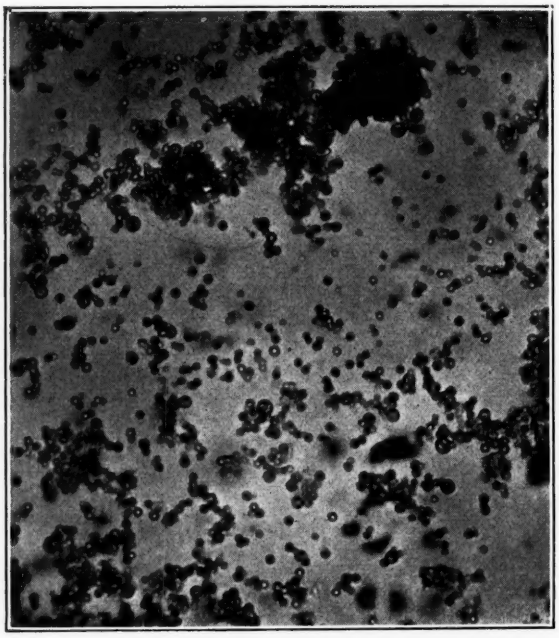

Fig. 1.—Photomicrograph of the exuvia which is found just inside the cocoon of the *Cecropia samia*. This consists of a skinlike mass, a large number of blood cells and a homogeneous fluid which make up the blood of the moth. This material may soak into the silk fibers and therefore may be considered of some allergenic significance. (×1014)

rosebushes in and around the city of Buffalo. While this moth belongs to a different species known as the *Cecropia samia,* it is closely related to the *Bombyx mori.* (Figs. 2 and 3.) These wild cocoons would obviously contain sericin entirely in its natural form. An extract of their silk cases may be said to contain the largest amount of silk glue or sericin.

Extracts of the various parts of these two species of cocoons were prepared in our allergy laboratory in the following manner:

(1) Pupal case of the commercial silk cocoon (*Bombyx mori*). Several of these pupal cases (the silk proper) were shredded finely and then

placed in sufficient buffered saline solution to be thoroughly soaked. Under a layer of toluol, extraction went on for three days. Sterilization was accomplished by Seitz filtration. The micro-Kjedahl method revealed a mere trace of nitrogen in the extract.

(2) The pupae of the above cocoons were first ground into a powder and defatted with ether, allowed to dry and then soaked with a buffered salt solution. A layer of toluol was added. Extraction was carried out for three days in the refrigerator according to Coca's method. The total nitrogen content amounted to 2.52 mg. per c.c.

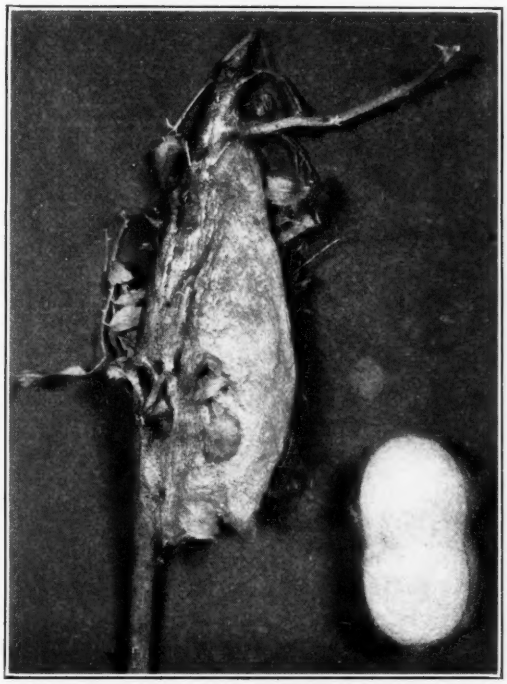

Fig. 2.—To the left is the cocoon of the moth, *Cecropia samia*, which is commonly found adherent to rosebushes. The other is the well-known silk cocoon, *Bombyx mori*. The latter appears cleaner because of the care which the cocoon raisers give it.

(3) The extract of the pupae of the raw Cecropia moth was prepared like that of the *Bombyx mori*. The total nitrogen was 1.93 mg. N. per c.c.

(4) The Cecropia pupal case (the raw silk) was also shredded and then extracted like the Bombyx silk case. The Cecropia silk absorbed less of the buffered saline solution than did the other. There was also considerable coloring matter, probably due to the sericin and other extraneous materials which had become adherent to the surface of the cocoon. The total nitrogen measured 0.05 mg. per c.c., a surprisingly low content.

(5) The exuvia from inside the Cecropia cocoon was treated with ether and then toluol. Extraction was done according to Coca's method for three consecutive days. Total nitrogen was 0.574 mg. N. per c.c.

(6) Commercial silk fiber, processed but undyed, was obtained from the Duffy Silk Company of this city. After shredding, an extract was made according to Coca's method. Repeated micro-Kjedahl tests failed to reveal any perceptible nitrogen.

Except extracts 1 and 6 which gave little or no nitrogen, the other extracts were diluted to 0.001 and 0.01 mg. of N. per c.c., respectively,

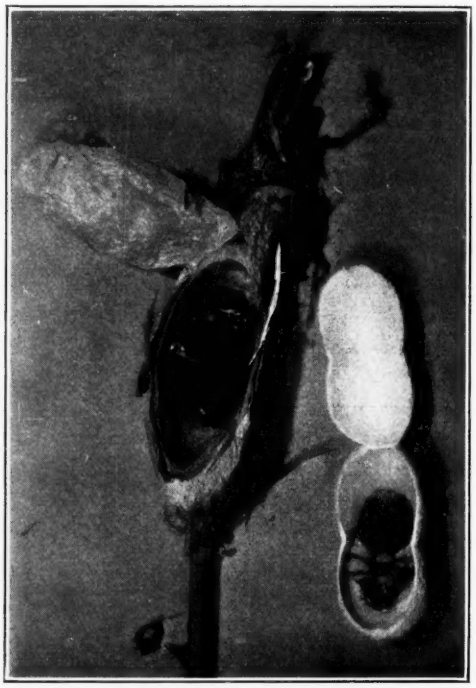

Fig. 3.—The same two cocoons shown in Fig. 2. The outer casing has been cut to show the walls and the pupa inside.

for routine use. Intracutaneous and scratch tests were made on six patients who were thought to be sensitive to silk. Two nonallergic young people were tested at the same time, as controls. Results of these tests are reported in Table II. Passive transfer tests were attempted but had to be given up when it was found that normal subjects also reacted to extracts of the pupae of both moths. Since silk has been classified also as a contactant, patch tests were made on all six patients with small quantities of the Japanese silk pupa (heated), the Cecropia pupa (raw, unheated), and commercial silk fiber such as is used in the manufacture of wearing apparel. Not a single positive reaction was recorded, even after allowing the contact to remain for seventy-two hours.

TABLE II

SKIN REACTIONS TO EXTRACTS OF DIFFERENT PARTS OF THE SILK COCOON AND ALSO COMMERCIAL SILK

PATIENT	CECROPIA PUPA (RAW SILK PUPA)		CECROPIA PUPAL CASE (RAW SILK CASE)		CECROPIA EXUVIA		BOMBYX MORI PUPA (JAPANESE TREATED SILK PUPA)		BOMBYX PUPAL CASE (HEATED SILK CASE)		COMMERCIAL SILK FIBER PROCESSED BUT NOT DYED	
	INTRA-DERM.	SCRATCH	INTRA-DERM.	SCRATCH	INTRA-DERM.	SCRATCH	INTRA-DERM.	SCRATCH	INTRA-DERM.	SCRATCH	INTRA-DERM.	SCRATCH
1. Mr. C. C.	++++	+	−	−	+++	+	++++	+	+++	+	−	−
2. Miss C. D'A.	++++	+	−	−	−	−	++++	+	−	−	−	−
3. Mr. E. R.	++++	+	−	−	+	−	++++	+	++	−	−	−
4. Miss M. S.	++++	+	−	−	−	−	++++	+	−	−	−	−
5. Miss M. W.	++++	+	−	−	−	−	++++	+	−	−	−	−
6. Mrs. C. N.	+++	+	−	−	−	−	++++	+	−	−	−	−
Controls (nonallergic)												
1. Miss J. T.	−	−	−	−	−	−	−	−	−	−	−	−
2. C. D'A.	++	+	−	−	−	−	++++	+	−	−	−	−

A study of Table II shows that all the patients reacted to the pupae (silkworm) of both moths. Two of them reacted to the exuvia and incidentally the same two reacted to the silk casing of the commercial cocoons. It readily became apparent that the active reacting principle was contained in the dead pupa inside the cocoon. Scratch tests were then made on one of the patients in this series. A small amount of the outer skin of the pupa, the inside of the pupa and the whole pupa *(Bombyx mori)*, respectively, were pulverized, and with addition of a drop of N/10 NaOH solution used in the usual scratch technic. All three gave positive reactions but the inside of the pupa gave the greatest response.

DISCUSSION

We agree with Coca and Milford[3] that the reacting principle of the cocoon is contained principally in its pupa. It is our theory that the pupa in its slow metabolism within the cocoon forms a histamine or a similar substance which accounts for the numerous but nonspecific positive skin reactions. Furthermore, these "false reactions" can be explained on the basis of a normal human reaction to a solution of the pupal proteins including its blood. This might be compared to the so-called normal reaction to horse serum. As in the example of horse serum, we occasionally find some one who may react to such an abnormally high degree as to manifest severe constitutional reactions. It can be reasonably asserted that the clinical experiences of Clarke and Meyer, and Figley and Parkhurst can be traced to an abnormal product in the silk pupa, rather than in the fiber or sericin. Commercial cocoons are killed by immersing them in hot water. In this way, it is thought that the sericin or silk glue is softened so as to permit easy unreeling of the silk fibers. It is possible that during this process enough of the pupal substance is allowed to soak into the silk fiber to account for the positive skin reactions obtained in our two cases and in those patients of the writers quoted above. In this connection, we wish to cite the significant observation which Ramazzini, the father of occupational diseases, made in the seventeenth century. Speaking of diseases known to silk workers, he said that "there are filaments (of silk) which have part of the bodies of the silkworms mixed with them. The workmen who draw them into threads . . . are usually troubled with a vehement cough and a great difficulty of breathing." An analogous situation arises in the ginning of cotton. We know that it is not the cotton fiber which excites allergic symptoms but the small particles of cottonseed which are unavoidably left in the fibers.

Can the specific reacting agent be contained in the exuvia which is found just inside the pupal case? The same two patients who reacted to the silk casing of the Bombyx cocoon, gave similar reactions to the

exuvia of the *Cecropia samia*. This exuvia may not be found in the Japanese cocoon, probably because the pupa may be killed before it has a chance to expel it. The epithelial structure and the blood cells and serum which make up this exuvial material strongly suggest the possibility that this possesses the reacting principle. More work needs to be done to substantiate this hypothesis.

We next considered the silk fiber and sericin which mainly make up the outer casing of the cocoon. It is well known that nearly all of the sericin is removed in the processing of silk thread. To disprove that the silk gum or sericin is the allergenic factor, we have seen that the raw, untreated silk casing of the *Cecropia samia* gave no reactions. Even the silk of the Bombyx cocoon which would contain more of the sericin than the processed silk fiber, gave rather weak reactions in only two of our patients. The contrast with the reactions obtained with extracts of the pupae is marked. Moreover, if we are guided by the nitrogen content of these extracts, it should be recalled that little or no protein is found in the extracts of the outer casings of both species of cocoons. In addition, the extract which was prepared from commercial silk (processed but not dyed) contained no nitrogen and gave consistently negative reactions. We cannot therefore subscribe to the theory that symptoms may be caused by the silk fiber alone.

CONCLUSIONS

1. Skin tests with an extract of the silk cocoon fibers and pupa were made on 166 current allergic patients. Fifty-six, or 33 per cent, of them gave positive reactions. Twenty-eight, or 27 per cent, of 102 nonallergic persons gave similar dermal response. Scratch and intradermal tests with silk extracts which were prepared by commercial and private laboratories gave practically identically positive reactions in our suspected cases of hypersensitiveness to silk. This unusually large proportion of positive cutaneous responses should not be laid to any particular technic of skin testing but rather to an incomplete and indefinite understanding of the silk allergen in the preparation of extracts.

2. Separate extracts were prepared of the various parts of the commercial silk cocoon and also of the *Cecropia samia* cocoon which is closely related to the former moth. Consistently positive reactions were obtained in cases of suspected silk hypersensitivity with an extract of the pupae (silkworm). Two of our patients reacted moderately to the raw cocoon silk and in like manner responded to the exuvial material which is found just inside the cocoon.

3. The commercial silk thread extract gave uniformly negative reactions in our supposedly silk sensitive patients and also in our nonallergic controls. Patch tests with the various parts of the cocoon

whether raw or in extract, failed to produce any skin reaction. We also failed to obtain any positive indication of skin sensitivity to the oil which is extracted by ether from the pupae.

4. The common but apparently unimportant skin reactions to routine silk extracts may be considered as a normal response to the proteins of the pupa which, like the sericin, are found in the fibers of the silk cocoon. Severe constitutional reactions, like the abnormal reaction to horse serum, may result from contact with this pupal material. Fortunately, this is a comparatively rare occurrence.

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BLOOD INORGANIC PHOSPHORUS IN ALLERGY*

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IT IS generally agreed that sensitiveness to a particular allergen, or the manifestation, such as asthma or hay fever, is more or less the end-result of the phenomenon of allergy. The more basic question is: What is the underlying constitution that allows the individual to become sensitized?

There is reason to believe that a clear understanding of this underlying basic factor might aid us in the management of allergic conditions with simplified therapy and more lasting results. In the attempts to discover such constitutional factors many theories have been advanced and some experimental work has been done. On the whole these efforts have been futile.

Among the various conceptions of the fundamental state underlying allergy the notion of a disturbance in the inorganic constituents of the tissues and blood has attracted wide attention. One of the most recent ventures has been the attempt to incriminate a phosphorus deficiency as one of the disturbances encountered in allergy. A disturbance in the blood phosphorus has been deemed reasonable because of the close relation between calcium and phosphorus metabolism and the numerous attempts to implicate calcium disturbances in the causation of allergy. In 1928, Sterling[1] reported inorganic phosphorus determinations of blood in 85 allergic individuals, of which 22 were suffering from hay fever, 12 from hyperesthetic rhinitis and the remainder from asthma. In only 17 of these 85 patients did he find a phosphorus value of over 3 mg. per 100 c.c. In 30 the phosphorus was below 1 mg., and in some there was no inorganic phosphorus found. There is no mention of the technic used in the determinations, whether they were made on serum or plasma and whether control tests were made on nonallergic individuals. On the basis of his laboratory findings Sterling added phosphorus and calcium to the therapy of his patients and states: "Hay fever patients and pollen asthmatics under our care last fall (1927) had absolute freedom from symptoms. . . . We think it was due to the addition of phosphorus and calcium medication."

On the basis of the above report one of us (S. M. F.) in 1929 made phosphorus determinations in 34 instances of hay fever, of which 10 patients were children. The lowest figure was 2.1 mg. and the average was 3.85. In 12 instances viosterol 250 D in doses of 30 drops

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daily was administered over periods varying from two to five weeks. There were no significant therapeutic results in the hay fever symptoms of any of the patients. In 4 cases in which viosterol had been administered subsequent phosphorus determinations showed no appreciable change. It is interesting to note also that some of the individuals who had a low phosphorus figure had excellent results with the regulation pollen therapy.

In 1931 we decided to reopen the subject for further investigation. The data cited are obtained from experiments made in 1931 and the early part of 1932.

Methods.—The inorganic phosphorus of serum was determined, usually in duplicate by the method of Fiske and Subbarow[2] (1925).

TABLE I

INORGANIC PHOSPHORUS OF BLOOD SERUM IN ALLERGIC INDIVIDUALS

NO.	NAME	PHOSPHORUS IN MG. PER 100 C.C. SERUM	NO.	NAME	PHOSPHORUS IN MG. PER 100 C.C. SERUM	NO.	NAME	PHOSPHORUS IN MG. PER 100 C.C. SERUM
				Adults				
1	J.L.	4.6	26	M.S.	3.0	51	L.D.	3.0
2	A.R.	3.4	27	M.A.	3.3	52	S.B.	3.1
3	U.S.	4.4	28	F.M.	3.6	53	E.C.	4.0
4	H.H.	3.4	29	L.C.	2.9	54	J.P.	3.6
5	C.	3.5	30	L.P.	3.6	55	J.E.	4.3
6	M.V.	3.4	31	A.B.	4.1	56	S.	5.0
7	S.Mc.	3.2	32	F.B.	3.0	57	T.	3.5
8	H.M.	3.4	53	L.O.	3.7	58	H.	5.0
9	L.K.	3.4	34	S.M.	3.1	59	C.	4.6
10	S.	3.4	35	H.W.	2.5	60	C.	3.7
11	D.H.	3.0	36	A.A.	2.9	61	J.I.	4.1
12	M.A.	3.0	37	A.C.	2.6	62	C.G.	5.1
13	E.D.	3.6	38	C.	3.5	63	L.S.	4.5
14	M.L.	3.5	39	Mc.	3.3	64	M.M.	3.8
15	S.	3.0	40	A.M.	3.4	65	C.L.	4.0
16	M.G.	4.1	41	R.C.	3.5	66	O.	3.7
17	C.N.	3.5	42	R.T.	4.6	67	S.M.	4.4
18	J.L.	3.0	43	C.	2.4	68	M.Mc.	4.2
19	D.	3.2	44	W.	4.0	69	I.B.	4.1
20	M.C.	4.6	45	M.R.	2.9	70	A.S.	3.3
21	E.R.	3.7	46	L.	3.3	71	S.L.	3.8
22	J.J.	3.8	47	B.B.	4.1	72	O.K.	3.2
23	R.	3.4	48	F.C.	3.3	73	S.	4.8
24	A.G.	2.9	49	G.S.	3.3	74	R.	2.9
25	E.F.	3.8	50	B.S.	2.7	75	L.	3.3
		Average phosphorus for adults 3.57						
				Children				
1	S.	4.5	7	W.C.	5.0	13	A.	3.8
2	C.S.	4.4	8	R.R.	4.4	14	L.	3.9
3	V.	5.2	9	V.S.	2.9	15		2.8
4	J.	5.9	10	T.M.	4.5	16		3.5
5	R.Mc.	4.9	11	P.U.	4.4			
6	G.	4.0	12	F.R.	4.4			
		Average phosphorus for children 4.28						

The inorganic phosphorus of a sample of plasma from the same patient was also determined in the majority of cases. We were surprised to find that in many cases the values obtained from samples of plasma were somewhat lower than those yielded from serum on the same blood sample. It was found that this was due to the sodium oxalate used as an anticoagulant. When oxalate was added to the blood sample in excessive amounts, no color development occurred in the filtrate, i.e.,

TABLE II

INORGANIC PHOSPHORUS IN BLOOD SERUM IN NONALLERGIC INDIVIDUALS

ADULTS						CHILDREN		
NO.	NAME	PHOSPHORUS IN MG. PER 100 C.C. SERUM	NO.	NAME	PHOSPHORUS IN MG. PER 100 C.C. SERUM	NO.	NAME	PHOSPHORUS IN MG. PER 100 C.C. SERUM
1	H.S.	3.8	12	I.R.	3.5	1	C.	5.2
2	T.	3.3	13	J.B.	3.4	2	L.E.	3.4
3	M.A.	2.8	14	A.G.	2.2	3	V.A.	2.9
4	M.M.	2.7	15	M.W.	3.0	4	M.K.	3.2
5	R.M.	3.1	16	F.C.	2.2	5	C.W.	4.8
6	J.Z.	2.8	17	M.Z.	3.8			
7	B.W.	3.0	18	B.B.	4.2			
8	B.W.	4.0	19	C.H.	4.1			
9	M.H.	3.8	20	J.S.	3.7			
10	A.M.	5.2	21	G.K.	4.0			
11	G.Z.	3.2	22	A.M.	3.5			
Average phosphorus for adults						3.42		
Average phosphorus for children						3.9		

TABLE III

INORGANIC PHOSPHORUS OF BLOOD SERUM

P (MG.) PER 100 C.C. SERUM	ALLERGIC ADULTS		CONTROL ADULTS		ALLERGIC CHILDREN	
	NO. OF CASES	PER CENT	NO. OF CASES	PER CENT	NO. OF CASES	PER CENT
2.0-2.5	2	3	2	9	0	0
2.6-3.0	15	20	5	23	2	12
3.1-3.5	23	31	6	27	1	6
3.6-4.0	16	21	7	32	3	19
4.1-4.5	12	16	1	5	6	38
4.6-5.0	6	8	0	0	2	12
5.1+	1	1	1	5	2	12
Totals	75		22		16	
Average phosphorus	3.57		3.42		4.28	

the sample would be taken as containing no inorganic phosphorus. Since these studies were made White and Monaghan[8] have shown that other anticoagulants, as well as oxalate, interfere with the determination of phosphorus. Because of these findings we are reporting only the phosphorus determinations on serum, which have been shown to represent "true" values.

Table I shows the individual determinations on a group of 91 allergic individuals, consisting of asthma, hay fever, hyperesthetic rhinitis and urticaria. In Table II are detailed the results in 27 other types of clinic patients. It will be seen that the figures in the two tables are similar. Table III shows a summary of the various groups and the numbers and percentage of cases to be found under each phosphorus figure. It will be seen that the figures for the allergic adults are very similar to those of the control cases in the adult group. The allergic children have higher phosphorus figures than the allergic adults, which is the usual difference in phosphorus between children and adults of a normal series. As compared with control children, the allergic children show a higher phosphorus figure, although it is very evident that because of the small number of cases in this control group any conclusions on this point would be extremely hazardous.

Our findings agree quite well with those of Lierle and Sage[4] whose paper in 1932 reports an average finding of 3.64 mg. of phosphorus per 100 c.c. of serum with variations between 2.4 and 5.1 in 16 asthmatics.

The most obvious interpretation of the discrepancy between our results and those of Sterling, since he did not report the methods employed is that the use of anticoagulants may have interfered with accurate determinations.

The information at present available warrants the conclusion that the level of inorganic serum phosphorus in the fasting allergic individual is within normal limits.

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185 N. WABASH AVENUE

HYPERSENSITIVITY TO BEES SUCCESSFULLY TREATED WITH WHOLE BEE EXTRACT

Case Report

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I REPORT the following interesting case of hypersensitiveness to bees because of the beneficial results which seem to have been obtained by applying the principle of desensitization.

The case is that of Mrs. H., aged twenty-seven years. She does not recall having been stung by a bee prior to 1927. At that time she married a man who earns his livelihood as a beekeeper. During the season of 1927, she was stung about fifteen times, she states, with the same effects experienced by the normal person. In 1928, however, when she was again stung by a bee, this was followed in a few minutes by difficulty in breathing and a little later by symptoms of urticaria. Thereafter, she noticed that she was very sensitive to bees. She found, for example, that if another person who had been stung on the finger by a bee, would rub his finger on her skin, urticarial wheals would develop. She also noted that when she came near an old hat of her husband that had not been used for several months but which contained many bee stingers, asthmatic symptoms would immediately develop. Whenever she happened to be stung, which occurred despite the utmost precautions, two or three times a season, she developed symptoms of sensitiveness—difficult breathing, wheezing, urticaria. Each experience seemed to be worse than the preceding one, developing more quickly and being of greater severity. The final experiences were of alarming intensity, and the patient lived in fear of being stung and of not being able to reach a physician in time to receive an injection of adrenalin.

The patient was supplied with ampules of adrenalin, and instructed to use them immediately following a sting. We were confronted with three possible means of solution: the husband could give up his business, or the wife could remove to other quarters during the time of the year when the bees were most active, or we could attempt to desensitize her.

We elected to try the latter. A quantity of live bees was sent to Dr. A. F. Coca at the Lederle Laboratories. He prepared extracts of the whole insects in the following dilutions: 0.00001, mg., 0.0001 mg., and 0.001 mg. N per c.c. The patient was tested intracutaneously beginning with the weakest dilution. She was found to react with

wheal showing irregular outline (pseudopod) and erythema to the 0.001 mg. but not to the 0.0001 mg., nor 0.00001 mg. dilutions. Treatment was begun June 2 with 0.1 c.c. of this dilution and repeated at seven-day intervals with gradually increasing doses until a dose of 1 c.c. of a 0.01 mg. dilution was reached, which since has been repeated at monthly intervals. An opportunity to test the efficiency of this treatment occurred in October, 1933, when she was stung by a bee. Following this, an area of redness about 6 inches in diameter developed at the site of the sting, and there was mild asthmatic breathing which, however, subsided without treatment. This attack was in no way comparable to the alarming symptoms which had developed prior to the treatment above described.

It seems reasonable to conclude that in this particular case the attempt to desensitize against bee sting has resulted in marked reduction of the sensitivity.

TRICOPHYTIN—ITS USE ACCORDING TO ALLERGIC PRINCIPLES*

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IT IS well recognized that papular, vesicular or bullous eruptions occur on the hands and other parts of the body coincidental with attacks of severe epidermophytosis of the feet.[1] Fungi are practically never found in these so-called "tricophytids" in my experience and in that of others.[2] The most plausible explanation of their genesis, the one rather generally accepted today,[3] is that they represent the violent reaction of a sensitized skin to allergens (fungi or their toxins) applied endogenously (or exogenously?). The absence of fungi is explained on the same basis as the absence of tubercle bacilli in the tuberculids—namely, that the degree of protective action of the skin, the allergy, is so high as to destroy the fungi that are deposited in or on it.

Many workers[4, 5, 6] who believe that this state of allergy is the cause of the tricophytids have made use of an extract of fungi, tricophytin, for diagnostic and therapeutic purposes. Although its value has not been definitely accepted by all, the general trend is toward its more frequent use.

The purpose of the present communication is to point out that which appears to be an error commonly occurring in the use of tricophytin and to present cases in substantiation of this thesis.

It is my observation both from a review of the literature and from observation of work in various American and European clinics that basic allergic principles are quite commonly violated in that for purposes of diagnosis and therapy the initial dosage is far too high. The rather common practice is to use a dilution of 1:10, 1:30, or 1:50[6, 7, 8] for diagnosis and for instituting treatment. One reason for this practice is the fact that such dilutions are recommended by the manufacturers of the various tricophytins in the instructions that accompany the packages.

Case 1. Dr. ____, aged thirty-eight years, a doctor of medicine, came under my care because of a very severe epidermophytosis of the feet and legs. Fungi were demonstrated in scrapings from the toes. After many months of futile treatment with various local applications it was decided to try tricophytin desensitization. Making the common mis-

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take, I injected 0.1 c.c. of a 1:30 dilution of trichophytin intradermally into the normal skin of the deltoid region. An enormous positive reaction appeared after twenty-four hours. At the same time his feet and ankles became worse. A total of three injections was given before we were forced to abandon such therapy because of the severity of the reactions. About eight months later it was decided to resume treatment with trichophytin. This time a dilution of 1:500 was used. Twenty-four hours later there was an extremely strong local reaction measuring 3×3 inches. Forty-eight hours after the injection there was an extremely acute exacerbation of the process on the feet, so severe as to necessitate the wearing of house slippers instead of shoes. About six weeks later local and focal reactions occurred from a 1:1,000 dilution. Because of the inconvenience from the focal reactions the patient feared to try a 1:10,000 dilution.

CASE 2.—This patient was treated by Prof. Kerl and Docent Dr. Urbach and is reported through their courtesy:

H. Q., aged twenty years, suffered from a vesiculobullous epidermomycosis of the feet with no lesions elsewhere. On April 26 she received an intradermal injection of 0.1 c.c. of 1:50 tricophytin. The next day there was a very strong reaction at the site of the injection. On April 30 it was noted that there had occurred an exacerbation of the process on the feet and that new vesicles were appearing on the malleoli and knees. Under soothing applications this exacerbation quieted down so that the patient was in a fairly quiescent state on May 5 when 0.1 c.c. of 1:200 tricophytin was injected intradermally. The next day there was a sharp exacerbation accompanied by a crop of tricophytids in a new location, i.e., on the palms of the hands. On June 6 a 1:500 dilution of tricophytin injected intradermally produced a strongly positive local reaction but no focal reaction.

In addition, four further cases have been observed but not reported since they are similar to the above. I have had a number of patients complain of some general malaise when the stronger dilutions of tricophytin were used. Upon reduction of dosage these symptoms disappeared. Sulzberger[6] has called attention to the local, focal, and general reactions occurring as a result of overdosage with trichophytin. He showed photographs of several such cases and warned against overdosage. However, he did not advocate a scheme of dosage but stated that his initial dose was 0.05 c.c. of the "standard concentration," equivalent in strength to a 1:30 dilution of the tricophytin of Metz. This I consider far too strong for routine use.

COMMENT

It is my belief that in testing or treating patients suffering from tricophytids one should test with serial dilutions of weak strengths and begin treatment with the weakest dilution giving a positive reaction.

A fair criticism of this plan is that inasmuch as there does not exist any method of standardization of tricophytin, the dilutions used at present under the name of "1:30," etc., might really represent much higher concentrations. The method of preparing tricophytin[8] shows that it would be difficult to standardize it on a basis of the relation of solid content to liquid; since it is an aqueous extract of fungi containing an unknown quantity of toxin. Dr. August Matras suggested that it might be possible to standardize tricophytin biologically, somewhat after the manner of standardizing tuberculin, by animal assay. The need for standardization is apparent.

CONCLUSIONS

1. When using tricophytin caution is necessary.

2. Cases with severe local, focal and general reactions to the orthodox, strong concentrations of trichophytin are reported.

3. The strength of tricophytin extract is unknown. An effective method of standardization would be a useful adjunct to the administration of this product.

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Department of Reviews and Abstracts

Selected Abstracts

Pyrethrum Sensitization. Its Importance and Relation to Pollen Allergy. Feinberg, Samuel M.: J. A. M. A. **102**: 1557, 1934.

In a series of 225 patients sensitive to ragweed pollen, 104, or 46.2 per cent, gave cutaneous reactions to extracts of commercial pyrethrum. In another series of 113 allergic patients, chiefly with asthma and hyperesthetic rhinitis who were not sensitive to ragweed, a 2+ reaction was obtained in only one instance.

It appears that sensitiveness to pyrethrum is a group sensitiveness. This is substantiated by the passive transfer of serum of a patient who gave good reactions to both ragweed pollen and pyrethrum into normal skin. Several of the sensitized sites were desensitized to pyrethrum, and when subsequently tested with ragweed extract, failed to give positive reactions.

The significance of this study is the fact that many ragweed sensitive individuals are susceptible to allergic attacks any time of the year as the result of exposure to pyrethrum products.

Desensitization to Insulin Allergy. Bayer, L. M.: J. A. M. A. **23**: 102, 1934.

A diabetic woman, aged forty-eight years, required insulin. Five units were taken once or twice a day for a period of ten days without allergic manifestations. Insulin was then stopped, and was not readministered for a year. Then 4 units twice a day, increasing to 14 units twice a day, did not completely control the glycosuria. There was a gradually increasing occurrence of wheals at the site of injection which persisted despite changing the brand of insulin. These wheals occurred not only at the site of injection but also over the remainder of the body, particularly at previous injection sites, and occurred from fifteen to thirty minutes after every dose. Insulin was stopped but reinjected one week later.

The first 5-unit dose was followed in twenty minutes by violent abdominal cramps, macular itching rash, and a sense of choking. Although there was no allergic family or past history other than hives in early childhood, skin tests of the various brands of insulin were done; all brands including beef insulin and pork insulin gave a large wheal.

A program of desensitization, planned to be effective in one day, was then undertaken. A brand known to contain both beef and pork was chosen, and injections with 1/100 units were begun. This proved too strong, and subsequent diminutions of concentration also proved too strong until but 1/1,000 of a unit could be tolerated. Injections were given at about one-half-hourly intervals thereafter. Reaction to increasing doses did not appear, and within six hours 5 units could be taken without any reaction whatever. The dosage was then increased to 10 or 15 units daily, which could be tolerated without any allergic symptoms. Although a few wheals reappeared from time to time after the injections, tolerance to insulin was enormously increased.

Allergic Reaction to Dinitrophenol. Frumess, G. M.: J. A. M. A. **102**: 1219, 1934.

A case is described of a definitely allergic individual who suffered at times with transient urticaria from given foodstuffs. She was given 0.1 gm. of the sodium salt of dinitrophenol twice daily. Seventeen days later she presented giant urticarial wheals of enormous size, some of them fully 8 cm. in diameter. The pruritis was intense. Her diet was restricted to beef and rice, but the symptoms persisted. One-tenth c.c. of the patient's serum was injected intracutaneously into the back of a nonallergic individual at two sites. The following day a small amount of powdered dinitrophenol was rubbed into a scratch over one site of the transferred serum with physiologic sodium chloride as a solvent. Within three minutes a typical wheal and flare reaction resulted. On the control site no reaction occurred. The urticaria and angioneurotic edema became gradually more intense. To a scratch on the forearm of the patient a small amount of dinitrophenol was applied in dilution of saline. A small wheal was produced, and there was an intense itching and flaring up of other lesions.

Local Cerebral Anaphylaxis in the Dog. Davidoff, L. M., and Kopeloff, N.: Proc. Soc. Exper. Biol. & Med. **31**: 980, 1934.

In 35 dogs, horse serum, egg albumen, and other allergens were applied in different animals to the brain through a craniotomy wound. No symptoms followed this initial application.

Several days later the animals were injected with the sensitizing substance. Within a few minutes definite weakness occurred on the opposite side of the body at sites corresponding to the motor areas of the brain to which the antigen had been applied.

From these findings it appears that local sensitization of cerebral tissue may occur by direct application of protein substance.

Experiments on Sensitization of Guinea Pigs With Simple Chemical Compounds. Landsteiner, Karl, and Jacobs, John: Proc. Soc. Exper. Biol. & Med. **31**: 790, 1934.

Guinea pigs rubbed repeatedly with salve containing p-phenylene diamine showed increased redness and scaling in comparison to controls. The same result was obtained by repeated intracutaneous injections of the saline solution of the same compound. Sensitization was likewise obtained by repeated injection of 0.02 mg. of this compound, as well as salvarsan and p-nitroso-dimethyl-analine.

About seven days after the twentieth injection, given at from one-day to three-day intervals, a number of the animals showed pinkish, more or less elevated, areas averaging 0.75 to 1.0 cm. in diameter within twenty-four hours.

Another substance to which these animals could be sensitized was dinitro-chlorbenzene. Suberanilic resorcine dye also gave reactions. Injections of aminoazobenzene, aminoazobenzene disulphanic acid, and the carbohydrate dextran have so far failed to do so.

Comparative Experiments on Local Anaphylaxis of Different Organs. Scholer, H.: Ztschr. f. Immunitat. **79**: 99, 1933.

Qualitative differences in the strength of a local anaphylactic reaction have been noted before by different experimentors. This qualitative difference has varied with the animal used, the method of injection, and the particular tissue chosen. In the present series of experiments, however, there is demonstrated the complete nonreactivity of a sensitized tissue. Rabbits were injected with horse serum (and some with swine serum). Sensitization was considered established upon the appearance of the Arthus phenomenon. Thereafter, injection of the antigen into the intestine or into the mesentery induced no inflammatory changes in the tissue. Injection of the antigen into the wall of the stomach elicited a strong positive reaction.

Further experiments, with Jequiritol No. 4 of Merck, showed that the negative results in the intestinal tissue were not due to a lack of capacity of that tissue to respond to inflammatory processes.

The conclusion is reached that an actual antigen antibody reaction took place in the intestinal wall which, however, called forth no anaphylactic symptoms in that tissue.

Society Proceedings

SOCIETY FOR THE STUDY OF ASTHMA AND ALLIED CONDITIONS

TENTH ANNUAL MEETING, ATLANTIC CITY, N. J., APRIL 28, 1934

An Address Commemorating the Tenth Anniversary of the Society for the Study of Asthma and Allied Conditions.* ROBERT A. COOKE, NEW YORK CITY.

The meeting of the Society for the Study of Asthma and Allied Conditions has special significance this year of 1934 in that we celebrate the tenth anniversary of the founding of the Society.

The idea that there should be a forum for presentation and discussion of cases and studies on allergy had been in the making for some time. It is my recollection that Dr. Harry L. Alexander was the originator of the plan, and several of us had talked the matter over on numerous occasions. These conversations culminated in a meeting on March 7, 1924.

I cannot do better than read the secretary's minutes of this meeting:

"An organization meeting of those interested in the study of asthma was held March 7, 1924, at Dr. Cooke's office, 375 Park Avenue, New York City. Those present were Drs. J. Alexander Clarke, Jr., Arthur F. Coca, Robert A. Cooke, Ruth Guy, Warfield T. Longcope, George Mackenzie, Edwin P. Maynard, Royce Paddock, Francis M. Rackemann, Oscar M. Schloss, Albert Vander Veer, Jr., and Harry L. Alexander. After statements concerning the purposes of the meeting, it was agreed that a formal organization be effected and that there be two officers—a president and a secretary-treasurer. Nominations were in order and Dr. Cooke was unanimously elected president and Dr. Alexander secretary-treasurer.

"It was moved and seconded that the name of the organization be 'The Society for the Study of Asthma and Allied Conditions.' Motion carried.

"It was moved and seconded that the president appoint a Membership Committee of three to recommend names of applicants to the Society. Motion carried.

"It was moved and seconded that a committee of three members be appointed to draw up a constitution to be presented to the Society for its consideration at its next meeting. Motion carried.

*Delivered at the dinner of the Society held at the Hotel Traymore, Atlantic City, April 27, 1934.

"It was moved and seconded that there be two meetings a year, one of these to take place in New York City, and that the next meeting be held at the Hotel Traymore, Atlantic City, Sunday afternoon, May 4. Motion carried.

"It was moved and seconded that the dues be two dollars a year. Motion carried.

"It was moved and seconded that a committee be appointed to communicate with the Society of Immunologists to establish relations with them. Motion carried.

"It was moved and seconded that an Executive Committee of three, with the officers of the Society ex-officio, be appointed by the president to undertake the functions of all committees heretofore passed upon. Motion carried.

"It was the sense of the meeting that this Society should be of particular service by studying problems of asthma and allied conditions that are relevant to public health rather than it be a medium for purely scientific discussion. It was agreed, however, that a suitable classification of asthma and the terminology of hypersensitiveness be considered.

"It was moved and seconded that each of the following problems be studied by those members of the Society who may be interested in one or more of them:

"1. The relation of infections of the respiratory tract to asthma.

"2. The classification of asthma and allied conditions.

"3. The terminology of hypersensitiveness.

"4. Investigation of industrial asthmas.

"5. Methods of preparation of materials for testing and therapeutic use.

"6. The investigation of asthma clinics.

"Motion carried. It was the sense of the meeting that each member of the Society should indicate which of the problems they were willing to study and that committees would be thus automatically formed. The meeting was then adjourned. Signed, H. L. Alexander, Secretary."

The first semiannual meeting of the Society was held at the Hotel Traymore, Atlantic City, May 4, 1924. There were seven members present. A Constitution and By-Laws were adopted, and thirty-one members were proposed and elected.

Since this date we have traveled on with no serious disharmony. There have been two meetings each year. The membership has increased to ninety-one. There are twenty states represented, together with the District of Columbia, Hawaii, and Canada. The meetings are well attended by members and guests.

Several times during these past years the question has arisen whether or not we should affiliate ourselves with the Association for the Study of Allergy. I believe I am correct in saying that it has been the sense

of this Society that we should not enter into an organizational union for the reason that the majority of members would not attend meetings far afield as would be necessitated by any union with the sister Society and would therefore be deprived of the privileges that membership in this Society sought to obtain.

The most cordial reciprocal relationship does exist in that the privileges of the meeting are always extended and attendance is cordially welcomed.

The gratitude that we feel and the felicitations that are extended on this our tenth birthday cannot help but be tempered by the sober second thought of how well we have fulfilled our self-imposed trust.

Let me take up here some of the objects as read in the minutes of our first meeting. "It was the sense of the meeting that this Society should be of particular service by studying problems of asthma and allied conditions that are relevant to public health rather than it be a medium for purely scientific discussion."

Except for the few statistics on asthma collected by Dr. Vander Veer and a paper by Dr. Gay on the economic aspects of hay fever, there has been no great effort along these lines. Perhaps asthma is not a sufficiently serious public health problem to attract attention, but certain it is that it is a condition in which the morbidity rate is high. Our clinical contacts assure us of this fact, but as yet no data have been collected to tell in clear figures either the frequency of asthma or the serious economic loss sustained by the victims and their families. I am sure that a concerted and well-directed effort along these lines would be of value to the public and to the profession, but preliminary work is necessary in order to convince any outside organization that financial support for a thorough investigation would be worth while. But since asthma and the other allergies are not communicable diseases, there would be little interest in such data unless it could be shown that there are sound preventive measures or that proper treatment requires facilities for special care not now readily available to the public. One very important step in this direction has been taken in the last two years by the establishment of a Standards Committee representing the two societies interested in allergy, of which I shall speak again. By the recognition and certification of clinics maintaining certain minimal requirements it should be possible in time to improve the facilities for the diagnosis and treatment of allergic diseases. Diagnosis, treatment, and prevention are all important problems and merit the best efforts we have to give.

What of the other problems enumerated in our first minutes?

On the relation of infections of the respiratory tract to asthma a great deal of work has been done. Although at the time this was suggested there was a feeling that infection played a part, today there is a definite consensus of opinion, and I might even say absolute proof, that there

is an important causal relationship and an improved therapeutic technic that is securing more than temporary relief to these patients that ten years ago were doomed to hopeless invalidism.

As to the classification of asthma and allied conditions and terminology of hypersensitiveness there have been contributions and perhaps a clarification of ideas, but even now I believe the time is not ripe for any attempt at concerted Society action. These are problems that will settle themselves only with a more thorough knowledge of basic facts regarding the mechanism of the various forms of the allergic reaction.

An investigation of industrial asthma as such seems to have interested no one particularly. These cases have no such importance as silicosis, asbestosis, and anthracosis, as asthma does not affect men generally in any dusty trade. The nearest approach to it is the castor bean sensitiveness, and even its frequency does not constitute a public health problem.

The methods of preparation of material for testing and therapeutic use have received attention, and I hope that the next few years will settle this problem, and the Society may then feel justified in accepting some system that may be used by all. It would be a great help.

Perhaps the most important work that we as a Society have undertaken with the co-operation of the Association for the Study of Allergy has to do with the standardization of allergy clinics. A Standards Committee of the two societies has been occupied with the problem of certification of allergy clinics. To date 34 have been recognized. Only a beginning has been made. How far we may go and what good we may do will depend upon the backing we receive from the medical fraternity and the methods we employ.

Our attempts at standardization of clinics should be and will be concerned solely with minimal requirements. We should lead rather than drive, educate rather than compel. This we can do by maintaining our own standards of excellence and integrity. I believe that every member of this Society is imbued with high ideals, and as a Society we can do no greater good than by maintaining such standards and exercising care in the selection of new members. This must all be done without a "holier than thou" attitude but with the humility becoming an inward feeling of rightness.

In 1928 the JOURNAL OF ALLERGY was started under the able editorship of Dr. H. L. Alexander, one of our charter members. It has been accepted as the official organ of our Society and is the medium through which most of the publications of members are presented to the medical world. It is an excellent Journal and should receive our hearty support.

A Society such as ours—such as any society of clinicians and scientists—is a loosely knit organization. We think of it as going smoothly if we stop to think of it at all. For that reason all such organizations

if they are to continue and be successful have to have a central and directing force—that force or heart of the society is not its membership no matter how select—it is not its president no matter how able—for he is temporary. This motivating force is its secretary. For nine years Dr. Spain has been our secretary. He has given unsparingly of his time and thought. I feel that this Society has great cause to give thanks for his able, sincere and successful direction for it is largely through him we have moved on so easily.

Looking back then over these past ten years I feel that we may felicitate ourselves for what we as a Society have accomplished. Our field of medical endeavor has been a relatively new one. We have accomplished some things but much remains to be done. Allergists as a class are not infrequently criticized by the medical profession. This has been caused partly by the unwarranted claims by those practicing allergy and partly by an expectation or hope on the part of our medical confreres that we could accomplish the impossible. As a group we must be conservative, sound, and sane. With our minds soaring high but with our feet planted solidly on the good earth let us proceed with our many problems, honestly and carefully searching for the truth. By such means only will we truly progress and assure our continued existence.

Pulmonary Lesions Resembling Pneumonia as the Result of Allergic Shock. G. L. WALDBOTT, DETROIT. (Abstract.)

The author quotes literature which suggests that what is known in animals as protracted anaphylactic shock also exists in man. The opinion is expressed that the chief pathologic exponent of this condition is a hemorrhagic edematous lesion in the lungs which has become infiltrated with leucocytes. He refers to two individuals in whom injections with horse hair and cotton seed extracts produced severe shock. After from eight to twenty hours, respectively, the clinical manifestations of a low grade bronchopneumonia ensued. Another such instance was observed in an asthmatic patient who developed shock following ingestion of 5 grains of aspirin.

In his series of 34 cases of so-called thymic death, a condition which, he believes, is identical with allergic shock, bronchopneumonia developed before death occurred in 10 cases. The clinical course corresponded closely with that observed in the above quoted cases of allergic shock. He describes in detail the cases of two children in whom ingestion of orange juice and milk is suspected of being the cause of shock. The autopsies revealed edematous areas in the lungs which showed leucocytic infiltration, particularly in their marginal parts.

A survey of 12 asthmatic children who had been hospitalized on account of pneumonia revealed that in 7 there was an afebrile period last-

ing from six hours to five days during which severe asthmatic wheezing was present. The average duration of the pneumonia in these cases was four days, the average white blood count 16,600, the average highest temperature 102.3° F. The course of the disease was much milder than in bronchopneumonia.

While it is impossible to rule out infections of atelectatic areas, the evidence brought out suggests that the cause of some cases of low grade pulmonary infections may be an allergic edema which may subsequently become infiltrated with white blood cells and possibly be infected.

DISCUSSION

Dr. Joseph Harkavy, New York City.—Dr. Waldbott's paper is very interesting and contains many phases which are difficult of interpretation. I think it would be wiser for the sake of clarity if we distinguished the various types of pulmonary reactions which can be attributed to allergic or anaphylactic shock.

There is no doubt that when you get a generalized allergic or anaphylactic shock in response to ingestion or injection of certain foreign proteins you may get a pulmonary reaction clinically and pathologically resembling bronchopneumonia. Such a picture characterized by marked congestion and edema in microscopic section is not necessarily specific of allergy, because you can find similar pathologic changes in acute pulmonary infections such as influenza, which many of us saw during the epidemic of 1918. Experimentally you may get pulmonary congestion, capillary thrombosis, cellular infiltration and atelectasis, as an anaphylactoid phenomenon following injection of arsenicals, barium, etc.

Since the microscopic picture of edema, congestion, and cellular infiltration as such is not specific of allergy, on being confronted with a pathologic section presenting such a reaction, we are justified in designating it allergic only if it can be directly correlated with a shock reaction resulting from injection or possibly ingestion of a known specific allergen.

While it is possible that the case of the child quoted by Dr. Waldbott may fall into this category some one may raise the question whether aspiration of milk might not have been responsible for the pneumonia which followed, rather than an allergic reaction. Inasmuch as there is no evidence of milk hypersensitiveness either by the history or tests, it may well be asked why a child who was a foundling probably brought up on cow's milk for a year, should suddenly succumb with pneumonia following the drinking of a glass of milk. On the other hand, it is clear that this child had an upper respiratory infection previous to the onset of pneumonia. It is, therefore, possible that this respiratory infection may have sensitized the child's lungs, which reacted in the form of a pneumonia. The drinking of milk may have been fortuitous.

Foci of infection in nose, sinuses, and lungs themselves in the form of unresolved interstitial pneumonia may act as sensitizing agents. In constitutionally allergic individuals they may induce a reaction characterized (1) by bronchospasm with an asthmatic seizure, (2) by a rapidly progressive bronchopneumonia with temperature, etc.

Another type of allergic pulmonary response in man is acute lobar pneumonia. Investigators such as Wadsworth, Lauche, etc., interested in this subject champion the allergic concept of lobar pneumonia, which presupposes that a shocking dose of pneumococci has been implanted in the lung of an individual partially immunized against the pneumococcus. They support this idea experimentally by producing lobar pneumonia in rabbits by preliminary immunization with pneumococci. Without such partial immunization, bacteriemia generally follows the introduction into the lung of virulent pneumococci.

Thus, experimentally and clinically we have accumulating evidence that a pneumonic involvement of the lung may be allergic in nature. Dr. Waldbott is to be complimented for drawing our attention to the type of pneumonia which follows allergic shock and which presumably represents a pulmonary response to protein hypersensitiveness nonbacterial in nature. His two cases of pneumonic reaction previously reported following injection of cotton seed and horse dander represent important evidence for his thesis.

Dr. Royce Paddock, Newark, N. J.—I should like to ask Dr. Waldbott about the cellular pathology of the lesions; and whether he could determine what part the eosinophiles played in the cellular reactions.

Dr. A. H. W. Caulfeild, Toronto.—I should like to ask Dr. Waldbott if he has distinguished between atelectasis (nonpostoperative in character) and pneumonia in some of these cases.

Dr. Waldbott.—I pointed out in my paper that on a clinical basis alone it is impossible to say whether these lesions are due to atelectasis resulting from blocking off of smaller bronchi with mucus or whether they are due to edema. I feel, however, that the evidence which I presented indicates that the infection is superimposed on edema and not on atelectasis. There is a question in my mind whether or not bacterial invasion takes place at all in these lesions.

In regard to the question of eosinophilia, in some of the cases of so-called "thymic" death there were occasionally eosinophiles in the lungs, but not in all. In those in which pneumonia was associated with edema no eosinophiles were present.

In regard to Dr. Harkavy's remarks, may I say that in the primary state of pneumonia and also in certain forms of tuberculosis, edematous lesions develop resembling those which I have shown here. Some believe that these lesions are an expression of bacterial allergy. While they may not be specific of allergic shock, they appear to be the most characteristic lesion in allergic shock following injections of antigen. These edematous lesions may become infiltrated with leucocytes, either in an effort of the organism to rid itself of the lesion or as the result of infection.

Observed Differences Between Giant (Trifida) and Dwarf (Artemisiaefolia) Ragweed Pollen. Selian Hebald and James H. Barnard, New York City. (Abstract.)

CLINICAL STUDY

It was found in clinical work with an experimental series of ragweed sensitive patients that extracts of three different lots of giant

ragweed pollen collected in different seasons could be given in large doses interchangeably. These extracts were all made at the same time and standardized on a protein nitrogen basis. Two different extracts of the dwarf ragweed pollen could also be given interchangeably, and patients receiving large doses of dwarf ragweed pollen in every case took an equal dose of giant ragweed without a constitutional reaction. Constitutional reactions were frequent when patients who had received large doses of giant ragweed were given a similar dose of low ragweed. The work has been repeated with two extracts of giant ragweed and two extracts of dwarf ragweed prepared at the same time from pollens from two widely different sources and standardized on a protein nitrogen content. A series of 50 ragweed sensitive patients by direct skin test using test solutions of 10, 100 and 1,000 protein nitrogen units, showed no difference in the size of the skin reaction between the giant and dwarf extracts. These patients were intensively treated in groups with the four extracts, and in every case a change was made with similar local reaction and no constitutional reactions from one giant extract to the other giant extract as well as from one dwarf extract to the other dwarf extract. On changing the patients from dwarf ragweed to giant ragweed with dosages of 10,000 units, no constitutional reactions have resulted, but the change from giant ragweed to dwarf ragweed has resulted in frequent constitutional reactions (42 per cent of 49 cases) as well as greater local reactions. These results indicate a quantitative but not qualitative difference between the 2 ragweeds only as far as the capacity to produce constitutional reactions is concerned. They appear to be identical in the reactive capacity by direct test on sensitive individuals.

IMMUNOLOGIC STUDY

The ragweed extracts used in the previous study were tested against ragweed sensitive serums by the method of passive transfer. Giant and low ragweed extract in varying dilutions produced identical skin reactions in sites sensitized with dilutions of a sensitive serum in the same donor, supporting the clinical results of the similar direct skin reactions reported by Dr. Hebald.

The two giant ragweed extracts in the same protein nitrogen unit dilution neutralized sensitive serums in the same donor equally. Also the two dwarf ragweed extracts equally neutralized sensitive serums in the same unit dilutions and in the same donor, but when the giant and dwarf ragweed extracts were compared by passive transfer on the same donor with the same serums, it was found that the neutralizing capacity of the dwarf was always much greater than that of the giant ragweed. This difference was of a quantitative rather than a qualitative nature, since sites made with the serum mixed with dwarf ragweed in vitro gave no reaction on test with giant ragweed, and when sufficient strength

giant ragweed had been added to serum in vitro, sites made with this mixture gave no reaction on test with dwarf ragweed. With the purified proteins prepared from giant and dwarf ragweed the same difference in neutralizing capacity was observed.

DISCUSSION

Dr. Milton B. Cohen, Cleveland, Ohio.—I think that both the Society and these gentlemen are to be congratulated on the fine piece of work which has been presented here this morning. To me it is always a pleasure to find that a man makes a clinical observation and then is sufficiently familiar with good methods which are available so as to study that observation and run it down as far as it is possible to do so at any given moment.

I do not know what all this means. Apparently they have shown that giant and short ragweed have the same antigenic material, but that in certain characteristics of the material, in the test tube at least, it takes more of one than it does of the other to neutralize a certain reactive capacity of a serum.

All knowledge ultimately, I suppose, has some use, and undoubtedly this will have some use when we know more. What I should like to know from these gentlemen is this: Do they have any ideas yet from the clinical observations of patients, as to whether they are better off using giant or better off using short ragweed in their treatment? Obviously, if you can get as good a result from a giant ragweed, and the chances of having local and systemic reactions are less, it would be to advantage to use the giant. If, on the other hand, the results are not so good, the reverse would be true. I should like to have an answer to that question, if it is possible.

Dr. W. C. Spain, New York City.—Many of us have been intrigued into making comparative studies of the two ragweed extracts over a number of years, but I do not believe that any of us have even suggested the possibility of differences such as Dr. Hebald and Dr. Barnard have shown so clearly this morning. In their paper they have demonstrated that although the antigenic material in the high and low ragweeds is essentially the same, yet the neutralizing or constitutional producing capacity of the low is many times greater than that of the high.

It is interesting to speculate on just what effects these findings will exert on our ideas of pollen therapy. Possibly among the attributes of the low is a greater immunizing capacity which would favor its utilization to a greater extent in treatment and possibly to the exclusion of the high. This possibility, I believe, these investigators are considering now.

Moreover, the weak antigenic effect of ragweed in lower animals may not be due equally to both ragweeds, as it is now considered. This, I understand, too, is being studied at the Roosevelt Hospital, and many puzzling constitutional reactions may be better understood once we know a little more about these differences.

Another result of their study which I think is of very practical importance to us is as to whether the quantity and quality of the antigenic material in ragweed is influenced perceptibly by the climate or by the characteristics of the soil in which it is grown. Many of us have often

been undecided whether to buy local pollen or to buy pollen from some distance. I believe that this study of theirs has pretty well settled that question in our minds.

DR. ROBERT A. COOKE, New York City.—There is one thing I should like to emphasize and that is, for those using or preparing their own extract, the great necessity to know whether they are using high ragweed pollen or low for their extracts. If they happen to have been using low in a series of cases, and they purchase high, then it is perfectly safe; on the other hand, if they happen to have been doing the contrary, using high one year, and purchase in the market a pollen which happens to be low, they are likely to have some general reactions, especially where the perennial treatment is being used.

DR. S. HEBALD.—In answering the question as to whether to use the high or the low ragweed extract in treatment, there is no doubt but that there is a group relationship between the two. The use of either one will protect patients having ragweed hay fever. It seems clear though from our observations that if you use low ragweed extract, maximum tolerance in many patients will be reached with a much smaller dosage than if you use the high ragweed extract.

As to whether the high or the low ragweed extract would give better clinical results, that part of the work is now being done. At our Allergy Clinic at Roosevelt Hospital we have divided many of our ragweed patients into two groups. One is being treated with pure high ragweed and another with pure low. We hope soon to be able to say whether the one or the other has given us better clinical results—or possibly from a clinical point of view there may be no genuine difference.

DR. WARREN T. VAUGHAN, Richmond, Va.—May I ask a question out of order?

As I recall it, similar work was done several years ago, I think by Dr. Brown, in which a patient was worked up to a top dose of one of the ragweeds and a change made to the other ragweed, without any trouble at all. I can see how if the first one was short and the change was made to the giant, you would have no reaction. I wonder whether Dr. Hebald recalls the work and which way the change was made, because, as I recall it, the conclusions were quite the reverse from the present.

DR. S. HEBALD.—I should like to answer Dr. Vaughan's question by saying that I looked up Dr. Brown's protocols and out of 31 cases which he changed, 14 were changed from high to low ragweed without producing a constitutional reaction. In his group the largest dosage reached before making the change was 0.04 mg. of total nitrogen. This is equivalent to about 1,250 units of protein nitrogen. You have noticed that the greater percentage of our patients received seven or eight times this amount before making the change.

General Anesthesia in Allergic Patients. A Review of 204 Cases of Tonsillectomy and Radical Antrum Operations. ROSE ANDRE AND R. CLARK GROVE, NEW YORK CITY. (Abstract.)

A group of 204 allergic patients who underwent tonsillectomy and radical antrum operations under general anesthesia during a period of

three years and a half were studied from the standpoint of administration of anesthesia, operative technic, and postoperative complications. It was found that: (1) General anesthesia is as safe in allergic patients, even the severely asthmatic, as in other patients, provided (a) the patients are properly selected and prepared, (b) an operative method is used which combines light anesthesia and carbon dioxide and oxygen hyperventilation with a minimum risk of pulmonary complications, (c) careful postoperative treatment is carried out with the assistance of an allergist physician. (2) Pulmonary complications were entirely absent in this series of 204 cases of tonsillectomy and radical antrum operations performed on allergic individuals. There was one death from operative shock in an inactive tuberculous asthmatic patient.

DISCUSSION

DR. HARRY P. SCHENCK, Philadelphia.—I want to congratulate Dr. Andre and Dr. Grove for their courageous study of the effects of general anesthesia in a group of allergic patients. The conclusion that general anesthesia is as safe in allergic patients as in other patients seems justified by these studies.

There is some danger, however, in fostering the notion that general anesthesia in allergic patients may be entrusted to average anesthetists and average operators. We must remember that the patients in this series were subjected to careful preoperative study including attention to drug sensitivity; there was refinement of surgical technic; anesthesia was skilfully administered with attention to the induction of proper basal anesthesia and to the induction of hyperventilation at the close of anesthesia.

I was very much interested in the anesthetics used, since basal anesthesia by means of barbiturates has lost some of its terrors since the introduction of picrotoxin as an antidote. Avertin was successfully employed so that it would appear that other members of the alcohol series such as di-vinyl-ether would be of value. Ethyl chloride was successfully used and therefore cyclopropane may have a place.

I do not wish to minimize the importance of this study which I feel to be a real contribution, but I do want to make a plea for local anesthesia in radical sinus surgery and in tonsillectomy in allergic patients over fourteen years of age. The age limit may be extended to sixteen or eighteen years in some instances, but my experience has been that allergic patients are unusually amenable to surgery of the upper respiratory tract under local anesthesia.

The Caldwell-Luc operation and the Ferris Smith procedure, both radical steps, are best performed under local anesthesia because this ensures an almost completely dry field, particularly in the Ferris Smith, and minimizes shock.

I dislike criticizing the other fellow's cases, but the single fatality in this series occurred in a patient who was obviously a bad risk. Hemorrhage was a complicating factor. Although this patient was a bad risk, I believe that unilateral operation under local anesthesia would have minimized shock and hemorrhage, and prevented a fatality. We first performed our Caldwell-Luc operations under general anesthesia and encountered marked shock. Bilateral operation under local anes-

thesia still produced shock. We finally used the unilateral operation, five to seven days elapsing between the two sides, and have found this most satisfactory.

I appreciate the courage and honesty of this paper very much.

DR. GROVE.—There are a few comments which I think the surgeon might add which Dr. Andre, being the anesthetist, should not take the credit for.

The three things of most interest in the paper I think are, first, the fact that at all times the surgeon has complete control of the throat. The mouth is open and the patient is not gagging. He can keep the throat clear and get a good view of the larynx, and at the end of the operation, as she said, the pharynx is quite clear and the danger of asphyxiation is certainly minimized.

The second phase of the operation is the lowering of the head, which may increase the oozing or bleeding, but you keep the nasopharynx and the throat lowered and thereby aspiration is cut down.

The third feature is the hyperventilation. We never use straight ether but always combined with nitrous oxide and oxygen, and at the completion of the operation rebreathe with carbon dioxide for three minutes.

A good many general surgeons at Presbyterian Hospital in New York City have done a bit of work along this line and have found that the hyperventilation minimizes the danger of pulmonary complications, especially atelectasis.

I agree with Dr. Schenk that our one fatal case might have survived under local anesthesia. We might have thrown out that one case, because it was done in the first two months before our technic was perfected, when we were young and feeling optimistic and we started operating on a good many asthmatic individuals that we might not now, looking backward, but Dr. Andre and I talked over the question of a bilateral Caldwell-Luc after the completion of the first side, and we decided that if we did just one side we might never see him again, so we did the second, and the other side was too much. He was the third patient within the series, and since that time our results have been much better, but he was a rather borderline case, so far as the chest part of it was concerned. He was supposed to have been a tuberculous patient and not any too good a risk. I had the support of the internists that he would go through it, but we have changed our ideas on these cases and probably would not operate now.

Coming back to the bilateral Caldwell-Luc, if it is done properly and quickly, I think the average asthmatic person will stand it just as well as the single operation if the general condition is good. In our experience, working along rapidly and carefully, you can do it just as well, and the second convalescent period is saved.

I have to disagree with Dr. Schenk on the local anesthetic. I think in our experience, and I have also visited a lot of clinics elsewhere, it is not so successful as general anesthesia for the Caldwell-Luc operation. The results in most of our own local cases do not compare with the general cases.

DR. A. H. W. CAULFEILD.—The man who prefers a local anesthetic thinks it is fine and the man who prefers a general anesthetic thinks it is fine, and I doubt if we will be able to settle that problem here.

I should like to ask one question of Dr. Andre, about the one case she wanted to discard, and who died: Would she consider him allergic because of his tuberculosis or from some other point of view?

One other comment occurs to me. With reference to the literature in connection with pulmonary abscess, it seems to me that a group of 204 cases is rather small from which to draw very large conclusions, but I should like to congratulate the authors of the paper.

DR. ROSE ANDRE.—In reference to Dr. Schenk's remark about local anesthesia, I appreciate it very much, and the main point to bring out is that in most of our operations we use general. In the cases with secondary operation, many patients had had tonsillectomy and other nose operations done previously, and in these a local anesthesia would have been very unsuccessful, we believe, because of the great length of time that we so often have to spend in completing the operation, when it is secondary.

In answer to Dr. Caulfeild's question, I wish to state the facts about the one case we have talked about discarding. This was a chronic infective asthma with a superimposed chronic pulmonary tuberculosis.

Correspondence

To the Editor,
Journal of Allergy,
St. Louis, Mo.

May 7, 1934

Dear Sir:

In the issue of the JOURNAL OF ALLERGY for March, 1934, there was published a paper entitled "Studies of the Anaphylactic Antibody" by Oscar Swineford, Jr., B.S., M.D., Instructor in Medicine at the University of Virginia School of Medicine. This paper has been awarded the John Horsley Memorial Prize in a slightly modified form under the title "The Specific Control of Experimental Serum Reactions."

The John Horsley Memorial Prize was established in 1925 by Dr. J. Shelton Horsley of Richmond, Virginia, in memory of his father. It is awarded on the basis of competition to graduates of the University of Virginia School of Medicine.

The paper is being reprinted in its modified form for distribution to a limited group.

I am,

Very truly yours,

EDWIN P. LEHMAN, M.D.,
Chairman of the John Horsley
Memorial Prize Committee.